A STUDY TO ASSESS ASSOCIATION OF α-ADDUCIN POLYMORPHISM IN ESSENTIAL HYPERTENSION AND ITS IMPACT ON RESPONSIVENESS TO HYDROCHLORTHIAZIDE IN PATIENTS ATTENDING HYPERTENSION CLINIC IN A TERTIARY CARE HOSPITAL

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CERTIFICATE

This to certify that the dissertation entitled **"**A Study To Assess Association of α-Adducin Polymorphism In Essential Hypertension And Its Impact on Responsiveness To Hydrochlorthiazide in Patients Attending Hypertension Clinic In a Tertiary Care Hospital" by the candidate **Dr. J. Divya John Stephy** for **M.D PHARMACOLOGY (Branch VI)** is a bonafide record of the research done by her during the period of study **(2013 -2016)** in the Department of Pharmacology, Government Kilpauk Medical College, Chennai – 600010.

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DECLARATION

I solemnly declare that this dissertation entitled **"A Study To Assess Association of α-Adducin Polymorphism in Essential Hypertension and its Impact on Responsiveness to Hydrochlorthiazide in Patients Attending Hypertension Clinic in a Tertiary Care Hospital"** was written by me in the Department of Pharmacology, Kilpauk Medical College, Chennai, under the guidance and supervision of **Prof. Dr. T. Aruna, M.D.,** Professor, Department of Pharmacology, Kilpauk Medical College, Chennai – 600 010.

This dissertation is submitted to **THE TAMILNADU Dr. M.G.R MEDICAL UNIVERSITY** Chennai, in partial fulfillment of the university regulations for the award of **DEGREE OF M.D PHARMACOLOGY (BRANCH - VI)** examinations to be held in **APRIL – 2016.**

Date :

Place : Chennai **Dr. J. Divya John Stephy**

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Degree for which submitted	:	Doctor of Medicine (M.D) in Pharmacology.
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Year	:	2013 -2016.

RATIONALE FOR UNDERTAKING THIS STUDY:

Essential hypertension is a multi-factorial disease caused by interactions between an individual's genetic makeup and the ambient environment. A personalized pharmacogenomic approach to uncover possible potential benefits of existent pharmacotherapeutic intervention is a novel and needed approach in Hypertension management.

Abnormalities of membrane sodium transport in the kidney play an important role in hypertension. Alpha-adducin (ADD-1) is a ubiquitously expressed cytoskeletal protein that appears to be involved in the distal tublular sodium ion transport in the nephrons. Cusi et al. reported that the Gly460Trp polymorphism of *ADD-1* was associated with a salt-sensitive form of hypertension. Salt sensitive forms of essential hypertension, i.e., carriers of 460Trp variant showed a greater fall in mean arterial BP than Gly460Gly homozygous wild type carriers after hydrochlorothiazide treatment in Caucasians.

Hydrochlorthiazide is a 1st line anti hypertensive agent recommended by JNC8 (2013-14) recommendations for management of Hypertension. Identification of genetic influence of Adducin-1 polymorphism can serve to identify thiazide sensitive patients in the initial stage of disease, making way for a personalised and

Pharmacoeconomic solution for both the patients and the Health care system.

However, subsequent study results have been inconsistent. Very few studies have been reported worldwide about its relation to hypertension. Therefore, this study was undertaken to examine whether the Gly460Trp polymorphism of *ADD-1* was associated with essential hypertension and its effect on responsiveness to hydrochlorthiazide in our population.

ABBREVIATIONS

EH – Essential Hypertension

ADD-1 – Adducin 1 or α-Adducin

ECFV – Extra Cellular Fluid Volume

ACEi – Angiotensin Converting Enzyme inhibitor

ARB – Angiotensin Receptor Blocker

CCB – Calcium Channel Blocker

JNC 8 – Joint National Committee 8

SNP – Single Nucleotide Polymorphism

HCTZ – Hydrochlorthiazide

CKD – Chronic Kidney Disease

CAD – Coronary Artery Disease

CVA – Cerebro-Vascular Accident

CLD – Chronic Liver Disease

NCC – Sodium Chloride Cotransporter

SHR – Spontaneous Hypertensive Rats

SGOT – Serum Glutamic Oxaloacetate Transaminase

SGPT - Serum Glutamic Pyruvate Transaminase

SBP – Systolic Blood Pressure

DBP – Diastolic Blood Pressure

MAP – Mean Arterial Pressure

NICE - National Institute of Health and Clinical Excellence

ATC – Anatomical Therapeutic Chemical classification code of drugs

DDD – Defined Daily Dose

V_d – Volume of Disrtibution

SLC – Solute Linked Carrier protein

RAAS – Renin Angiotensin Aldosteone System

SHR – Spontaneous Hypertensive Rats

MHS – Milan Hypertensive Rats

LD_{50} – Median Lethal Dose

NOAEL – No Observed Adverse Effects Level

OPD – Out Patient Department

BMI – Body Mass Index

CBC – Complete Blood Count

RFT – Renal Function Test

LFT – Liver Function Test

FLP – Fasting Lipid Profile

RBS – Random Blood Sugar

EDTA – Ethylene diamine tetraacetic acid

ARMS PCR – Amplification Refractory Mutation System Polymerase Chain Reaction

SPSS – Statistical package for Social Sciences

ANOVA – Analysis of Variance

CONTENTS

A STUDY TO ASSESS ASSOCIATION OF α-ADDUCIN POLYMORPHISM IN ESSENTIAL HYPERTENSION AND ITS IMPACT ON RESPONSIVENESS TO HYDROCHLORTHIAZIDE IN PATIENTS ATTENDING HYPERTENSION CLINIC IN A TERTIARY CARE HOSPITAL.

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Year : 2013 -2016.

ABSTRACT

INTRODUCTION:

Essential hypertension is a complex polygenic inherited disorder. Abnormalities of membrane sodium transport in the kidney play an important role in hypertension. Alpha-adducin (ADD-1) is a ubiquitously expressed cytoskeletal protein that appears to be involved in the distal tublular sodium ion transport in the nephrons. Cusi et al. reported that the Gly460Trp polymorphism of *ADD-1* showed a greater fall in mean arterial pressure than Gly460Gly homozygous wild type carriers after hydrochlorothiazide treatment in Caucasians.

However, subsequent study results have been inconsistent. Very few studies have been reported worldwide about its relation to hypertension. Therefore, this study was undertaken to examine whether the Gly460Trp polymorphism of *ADD-1* was associated with essential hypertension and its effect on responsiveness to hydrochlorthiazide in our population.

METHODOLOGY:

This study was conducted in the Hypertension Out-patient clinic of Government Kilpauk Medical College during the period of June, 2014 to June, 2015 after obtaining permission from the IEC, GKMC. Open labeled, stratified randomized, parallel assignment, interventional safety and efficacy study of T.Hydrochlorthiazide 12.5mg once/day as an add on therapy in uncontrolled

(BP ≥140/90mmHg) Essential Hypertension with post hoc Genotyping of ADD-1 Gly460Trp by tetra-ARMS PCR assay was carried out among 100 Essential Hypertension patients.

RESULTS & CONCLUSION:

Three genotype classes were identified in our population: Gly460Gly (Homozygous), Gly460Trp (Heterozygous) and Negative (neither Homozygous nor Heterozygous).The prevalence of ADD-1 Gly460Trp mutation in our study group was 13%. Gly460Trp carriers showed significant linkage with higher staging of Essential Hypertension in spite of more than one class of pharmaco-therapeutic intervention.

Irrespective of genotypic variations in ADD-1, Hydrochlorthiazide 12.5mg once/day addition to the other class of antihypertensive agents produced additive antihypertensive efficacy of the regimes used. Gly460Trp was a low frequency genotype trait and did not show statistically different endpoint result variations in comparison to the other 2 genotypes (Gly460Gly & Negative) in our study.

INTRODUCTION

Essential hypertension (EH) is a typical example of an “iceberg” disease. A substantial proportion of the general population remain below the waterline with regards to diagnosis and adequate treatment. In spite of being an under reported and inadequately treated disease, its prevalence among 35-70 year old Indians is reported to be about 30.7%.[1] Hypertension causes 8.75% of annual mortal events in India, i.e., about 1.07 million deaths per year.[2]

Essential hypertension (EH) is a polygenic hereditary disease and its phenotypic expression is influenced by lifestyle and environmental factors. These lifestyle and environmental factors include an individual’s dietary hygiene (daily salt intake, saturated fat and dietary fibre consumption), level of physical fitness, alcohol intake and tolerability to psychosocial stress. Most of these lifestyle and environmental factors are grouped under modifiable risk factors.

While titration of blood pressure is possible by positively controlling an individual’s modifiable risk factors by means of health promoting non-pharmacological interventions, the inherent genetic risk of development and propagation of hypertension and its complications remains non-modifiable.

Renal handling of sodium salt has been ascertained to play a key role in the disease process. Any insult that impairs the physiological sodium balance in the body, either by affecting sodium retention or excretion reflects on the blood pressure of an individual.

Three mechanisms have been postulated to explain the renal mediated alterations in the internal sodium milieu.[3]

1. Limited sodium excretion due to a reduced Glomerular Filtration Rate
2. Any hormonal (e.g.: Angiotensin II, Aldosterone, Vasopressin, Endogenous Ouabain, Dopamine,etc) or genetic disorder that promotes sodium reabsorption in the distal nephrons
3. Renal ischemia, Oxidative stress and inflammation

Sodium is the major extracellular cation in the body. Increased retention of sodium salt results in an expansion of the extracellular fluid volume (ECFV). This expansion in ECFV results in structural and functional adaptations in the Heart, Brain, Kidney and Peripheral arteries. Hypertension is considered as an independent predisposing risk factor for congestive cardiac failure, coronary artery disease, cerebro-vascular accidents, renal dysfunction and peripheral arterial disease.[4]

Understanding the complexity of its etio-pathogenesis, makes the pharmacotherapy of EH simpler. Major classes of antihypertensive agents that are used in clinical practice can be broadly classified into:

I. Diuretics : Thiazide/ Loop/ K^+ sparing agents

II. Sympatholytics: α-Blockers/ β-Blockers/ Mixed α&β-Blockers/Central α2 agonists

III. Vasodilators: Calcium Channel Blockers/ K^+ Channel Openers/ Direct arterial vasodilator/ Mixed arterial & venous dilator

IV. Renin Angiotensin system blockers: Direct Renin Inhibitors/ Angiotensin Converting Enzyme Inhibitors/ Angiotensin 1 Receptor Blockers/ Aldosterone antagonist

Most antihypertensive agents modulate blood pressure by interfering with the determinants of blood pressure.[5]

Fig.1: Drugs modulate determinants of Blood pressure and modulate Blood Pressure. (Adapted from Principles of Pharmacology by David E.Golam

current treatment guidelines as per Joint National Committee 8 (JNC 8) recommends initiation of pharmacotherapy in patients < 60 years to achieve blood pressure goals of Systolic/Diastolic BP <140/90mmHg.[6]

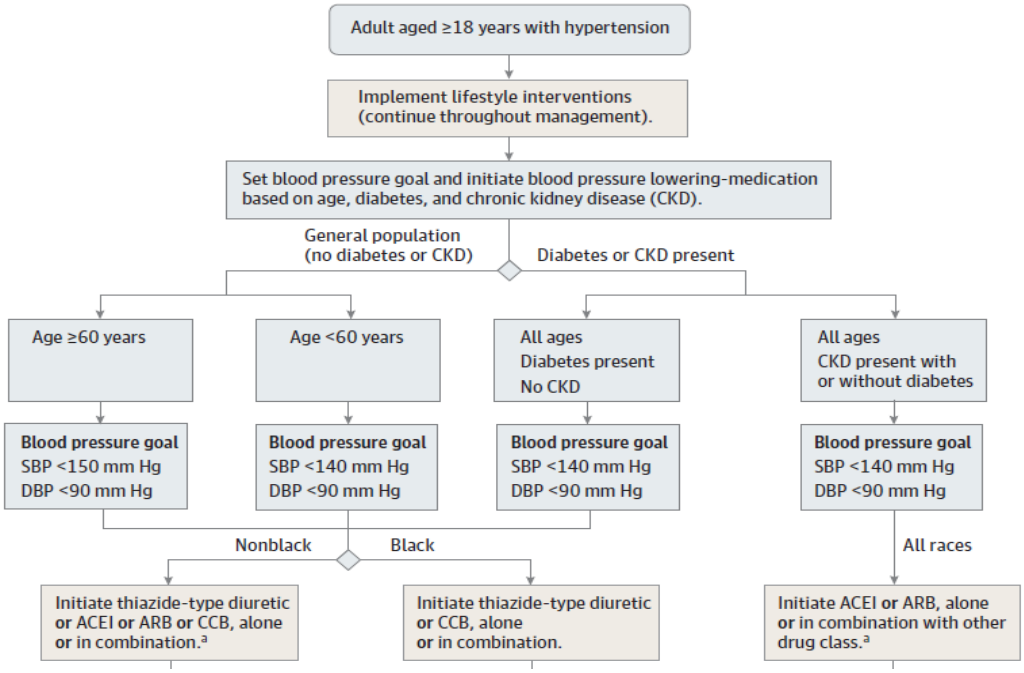


Fig. 2: JNC 8 guidelines in management of Hypertension.

Though a wide array of drug moieties are available, their extent of lowering blood pressure remains highly unpredictable. Evidence based JNC8 guidelines recommends the administration of thiazide diuretics / Angiotensin Converting Enzyme inhibitors/ Angiotensin Receptor Blockers(ACEi/ARBs) / Calcium Channel Blockers(CCBs), either combined or alone as the first line antihypertensive agents. But, ethnic and inter-individual variability in pharmacological response is seen with all the first line drugs.[7]

Dose- response relationship of antihypertensive agents follow either linear (adrenergic receptor blockers and CCBs) or non-linear patterns(ACEi/ARBs).[8] So in order to titrate the blood pressure

throughout a 24 hour period, an appropriate drug at the right dose and correct dosing regimen should be customised for each patient. This rational pharmaco-therapeutic approach is further challenged by the possible Pharmacokinetic/ Pharmacodynamic drug interactions due to other co-administered drugs, especially in geriatric population with polypharmacy.

Furthermore, the mapping of the human genome, genetic predictors especially single nucleotide polymorphisms have been identified as potential targets for affecting drug response.[9] SNPs involving Renin Angiotensin Aldosterone system, Adrenoceptors, Renal epithelial solute transporters have been brought to light.[10] Hence the concept of "Personalised Medicine"- Using knowledge of an individual's genetic profile to guide therapeutic decisions in diagnosis, treatment and prevention seems to be a clinching approach.

Adducin is a ubiquitously present tetrameric cytoskeletal protein made up of an α and either a β/γ heterodimeric subunits. It is coded by 3 genes ADD-1(α), ADD-2(β) and ADD-3(γ). It is involved in inter-cellular contact, signal transduction and cell membrane ionic transport. By means of positional cloning, α Adducin has been mapped at chromosome 4p16.3[11] In rats and humans, a SNP(Gly460Trp polymorphism) in α-Adducin leads to stimulation of Na+/K+-ATPase activity in renal tubular

cells resulting in increased sodium reabsorption and hence salt sensitive form of EH.[12]

In salt sensitive forms of EH in Caucasoid ethnic groups, carriers of Gly460Trp genotype variant showed a greater response (i.e., fall in Mean Arterial BP) when treated with hydrochlorthiazide than their counterparts with Gly460Gly homozygous wild type carriers.[13] However, subsequent study results have been inconsistent. Very few studies have been reported worldwide to establish association of ADD-1Single nucleotide polymorphism and Hydrochlorthiazide (HCTZ) responsiveness in Essential hypertension. Therefore, we have planned to determine whether the Gly460Trp polymorphism of *ADD-1* was associated with EH and its effect on responsiveness to HCTZ in our population.

AIM OF THE STUDY

Single nucleotide polymorphism involving the α-Adducin gene (ADD-1) has been identified as a possible genetic marker for the prevention, diagnosis and treatment of Essential Hypertension. Gly460Trp polymorphism of ADD-1 gene is associated with salt-sensitive form of hypertension, more amenable for treatment with thiazide diuretics due to augmented blockage of renal tubular sodium reabsorption.

With the basis of this hypothesis, the current study was undertaken with the following Aim:

1. To find the prevalence of α-Adducin polymorphism in patients with essential hypertension
2. To find if an association exists between ADD-1 Gly460Trp polymorphism and stage of Blood pressure control in subjects with their existing drug treatment
3. To assess the responsiveness to Hydrochlorthiazide 12.5mg Once daily (as an add on agent) among patients with poor response to their current antihypertensive treatment
4. To ascertain/ refute α-Adducin polymorphism mediated hydrochlorthiazide responsiveness among the subjects.

REVIEW OF LITERATURE

THIAZIDE DIURETIC AS AN ANTIHYPERTENSIVE AGENT - A PAST TO PRESENT PERSPECTIVE:

The modern era of antihypertensive drug therapy began with the break through discovery of an orally effective diuretic - Chlorthiazide by Beyer and Sprague in 1958.[14] The drug was synthesised by chemical modification of sufonamide compounds in search of diuretics with Carbonic Anhydrase inhibiting property. Serendipitously, the drug was recognised as a blood pressure lowering agent when it was used in patients with congestive heart failure.[15]

Following this observation, Clinical trials were undertaken simultaneously by two separate groups of researchers: Freis et.al and Beam et.al. Clinical experience by these pioneer researchers, proposed that the drug was equally effective and better accepted by hypertensive patients than a strict salt restricted diet.[16] Thiazide, when used in combination with the other classes of antihypertensive agents was also found to enhance their efficacy, down titrate the dosage required and ameliorate toxicity. Hence thiazide diuretics were published as adjunctive agents in management of hypertension in the mid 19th century [17]

Veterans Cooperative study, conducted in 1964, was a landmark randomised controlled clinical trial in the history of hypertension management. This trial established a superior survival benefit attributed by a cocktail of thiazide diuretic, reserpine and hydralazine among moderate to severe hypertensive patients.[18]

Subsequently, many clinical trials were undertaken to confirm the efficacy of thiazide diuretics as an antihypertensive agents. But all these trials recorded a reduction in diastolic blood pressure as the primary end point and concluded that a reduction of DBP by thiazide based cocktail of drugs produces cardiovascular survival benefit.[19]

So, clinical trials concentrating on reduction of Systolic Blood Pressure were undertaken, namely – SHEP (Systolic Hypertension in the Elderly Program) and ALLHAT (Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial). The ALLHAT trial was the largest RCT on hypertension management, and it compared the efficacy of initiation therapy with the various available antihypertensives on cardiovascular survival benefit.[20]

Combining results from these above trials, thiazide diuretics were recommended as first choice treatment for hypertension in the Seventh Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7)[21]

CURRENT STATUS OF THIAZIDE DIURETIC IN HYPERTENSION MANAGEMENT AMONG INDIAN POPULATION:

The antihypertensive pipeline has remarkably expanded over the last 60 years. Currently an appropriate Pharmaco-therapeutic intervention could be selected rationally from the abundant armamentarium of different classes of antihypertensive drugs available.

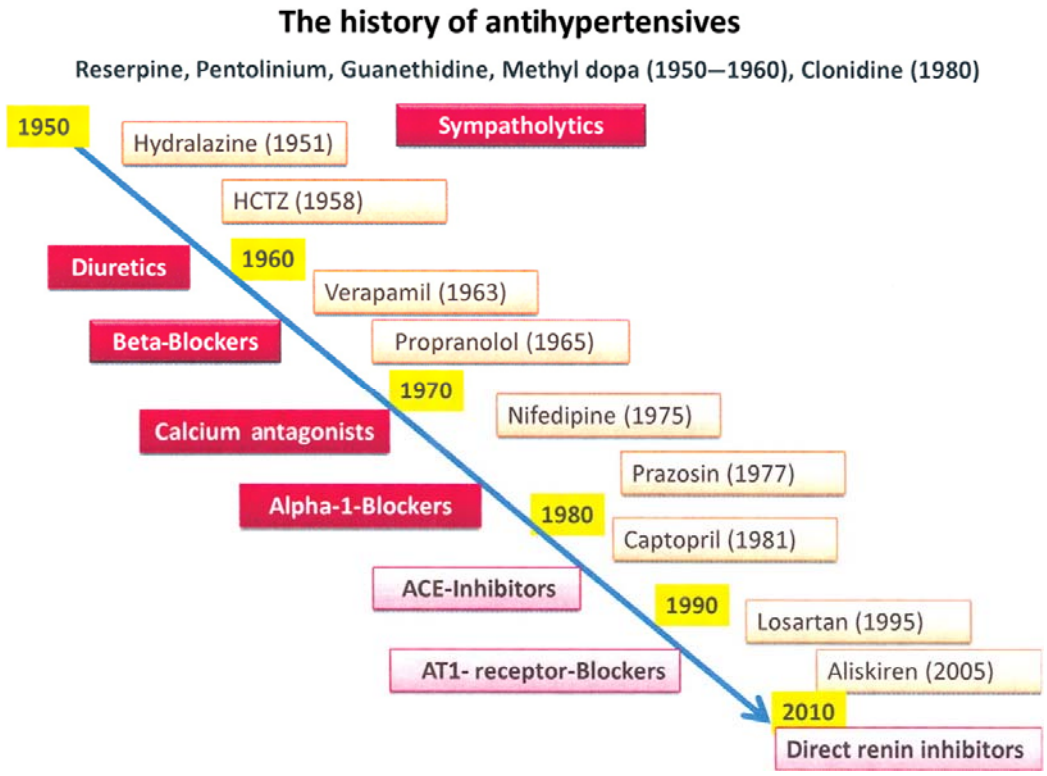
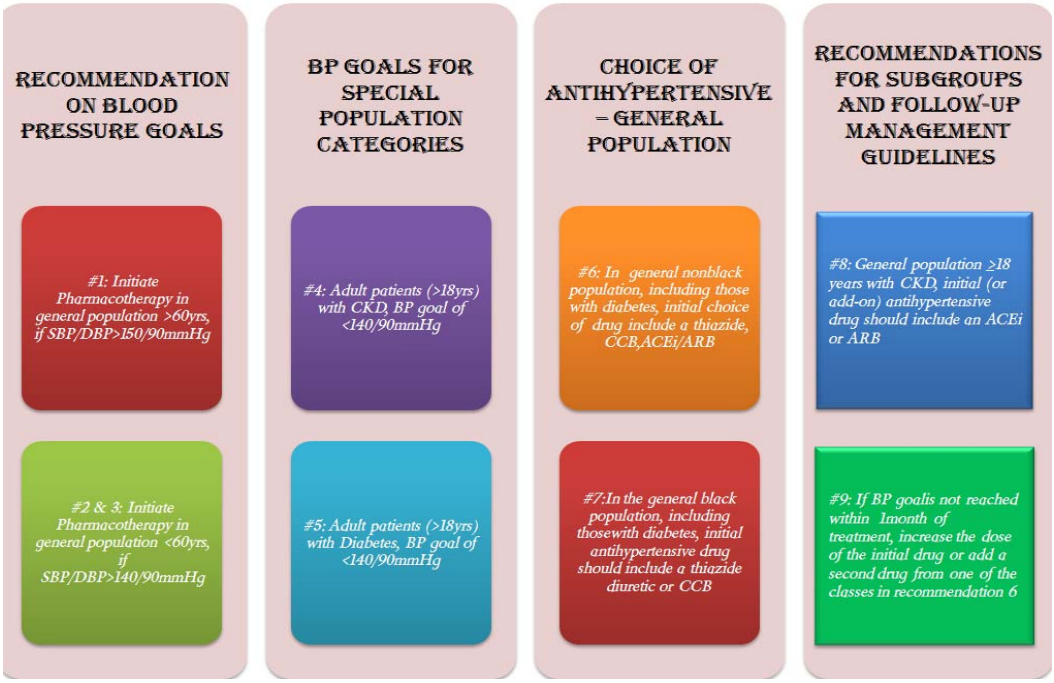


Fig.3: Timeline of discovery of Antihypertensive classes of drugs

The antihypertensive pipeline has evolved rapidly, probably with the understanding of various facets of hypertension pathophysiology and improvements in the field of science and technology. With this better

outlook on the disease process, different classes of pharmacological agents have been discovered and successfully used in clinical practice. The paramount role played by thiazide diuretics as one of the first step agent in the management of Hypertension still holds good to current date. The general schema for management of hypertension, according to JNC 8 recommendations is as follows:



Incorporating these recommendations to our Indian subpopulation, the following generalisations and dictums have been put to practice:

1. South Asian general population respond to antihypertensive management in a similar fashion like Caucasian population.[22]

2. Diuretics are first line agents in the management of hypertension in general population unless there is an absolute or relative contraindication to the specific drug per se.[23]

3. The 2011 update on hypertension management released by the National Institute of Health and Clinical Excellence (NICE) in India also Thiazide diuretics as first step pharmacotherapeutic agents, since they confer cardiovascular survival benefit.[24,25]

4. All the landmark studies establishing Thiazide diuretic as first step agent was conducted using Chlorthalidone. Effectively Equivalent dose of Hydrochlorthiazide (HCTZ) has been established from various studies. 25mg Clorthalidone = 50mg HCTZ. [26]

5. Among the Thiazide like diuretics, Hydrochlorthiazide is the most available and extensively used agent in India.[25]

UNDERSTANDING HCTZ AS AN ANTIHYPERTENSIVE AGENT:

Hydrochlorthiazide is a prototype member of thiazide class of diuretics. Its chemical formula is $C_7H_8ClN_3O_4S_2$ [27]

ATC Code	Name	DDD	Route of administration
C03AA03	Hydrochlorthiazide	25mg	Oral

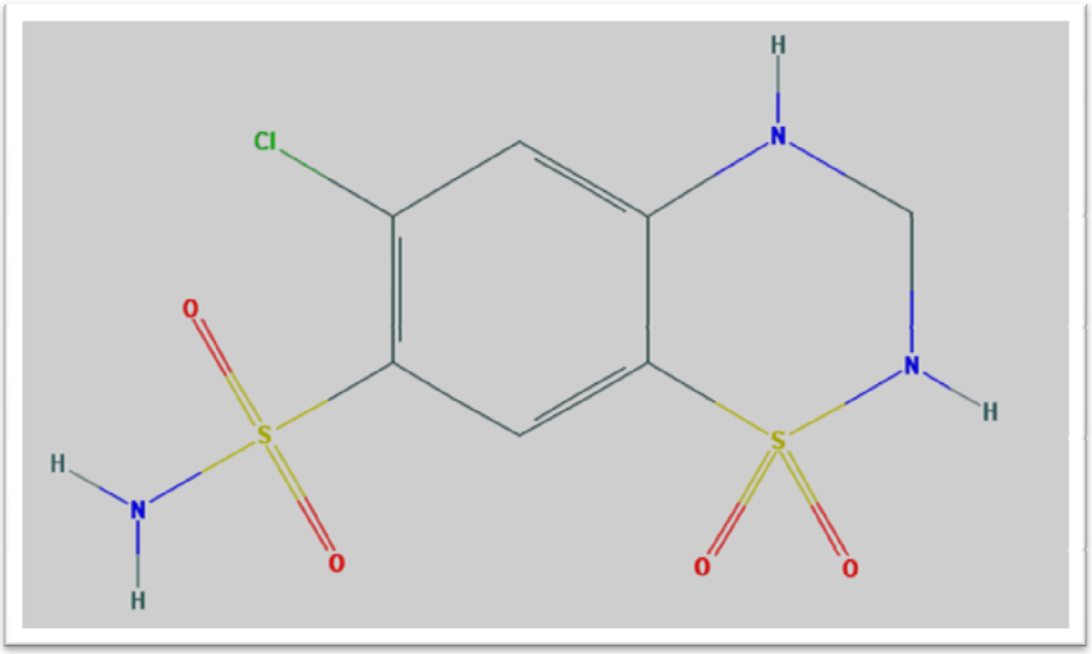


Fig.4: 2-D structure of Hydrochlorthiazide

Pharmacokinetic parameters:

Absorption	50-60% biovailability with orally administered tablet formulations
Distribution	V_d – Not available Protein binding : 67.9%
Metabolism	Not metabolised
Elimination	Renal elimination Does not cross blood brain barrier. Crosses placental barrier. Excretion in milk (+)
Half life	5.6 – 14.8 hours

Recognised targets of drug action:

- Inhibitor at Solute carrier family 12 member 3 – Na^+/Cl^- cotransporter [28]
- Inhibition of carbonic anhydrase type- 1,2,4,9,12 [29]
- Unknown action on Calcium-activated potassium channel subunit alpha-1[30]

Mechanism and site of action:

Micropuncture and in situ microperfusion studies have clearly demonstrated that HCTZ exerts its diuretic action by inhibiting Na^+/Cl^- cotransporter protein (NCC; gene symbol SLC12A3) in the distal convoluted tubules.[31]

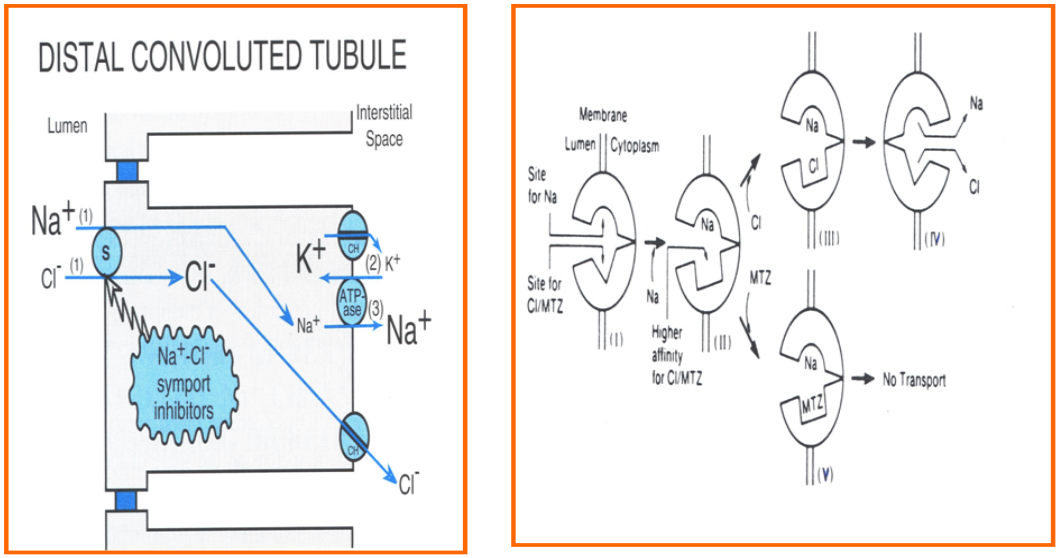


Fig.5: Mechanism of Action of HCTZ

The NCC reabsorbs Na^+ from the distal convoluted tubules (DCT) back into the interstitium and accounts for approximately 7% of total sodium reabsorption.[32] Under normal conditions, the NCC transports sodium and chloride from the tubular lumen into the epithelial cell lining of the DCT. This energy dependant process is supported by the sodium gradient established by Na^+/K^+ATPase activity in the basolateral membrane.

Since Thiazide diuretics inhibit the NCC, they decrease sodium reabsorption and increase fluid loss in urine. This volume loss through urine, helps in clearing off excess ECFV and Plasma volume, especially in volume overloaded conditions.

Volume loss also causes decrease in venous return, increase in renin release, reduction in cardiac output and reduction in blood pressure.[33] An acute transient effect of increase in total peripheral resistance within a few days of administration of Thiazide is noticed. This is due to compensatory release of sympathetic nervous system and renin-angiotensin- aldosterone system in response to the decrease in Cardiac Output.[34]

But on chronic administration of Thiazide diuretics, blood pressure reduction occurs in spite of full recovery of ECFV within 4-6weeks of Thiazide initiation.[35] If the antihypertensive effect of Thiazide diuretics is due to its diuretic/sodium depletion action alone, then loop diuretics should have superior blood pressure lowering efficacy. But in real time scenario, loop diuretics do not reduce blood pressure to the degree of Thiazide class of drugs.[36]

Hence the exact mechanism of blood pressure lowering of thiazides is probably not thru its diuretic/sodium depleting activity alone. Other probable mechanisms to explain the antihypertensive property of thiazides have been put forth, but still stands to be fully validated and understood.The hypothesis of the 'vasodilator theory', which highlights the response following the decrease in total peripheral resistance with Thiazide class of diuretics.[37]

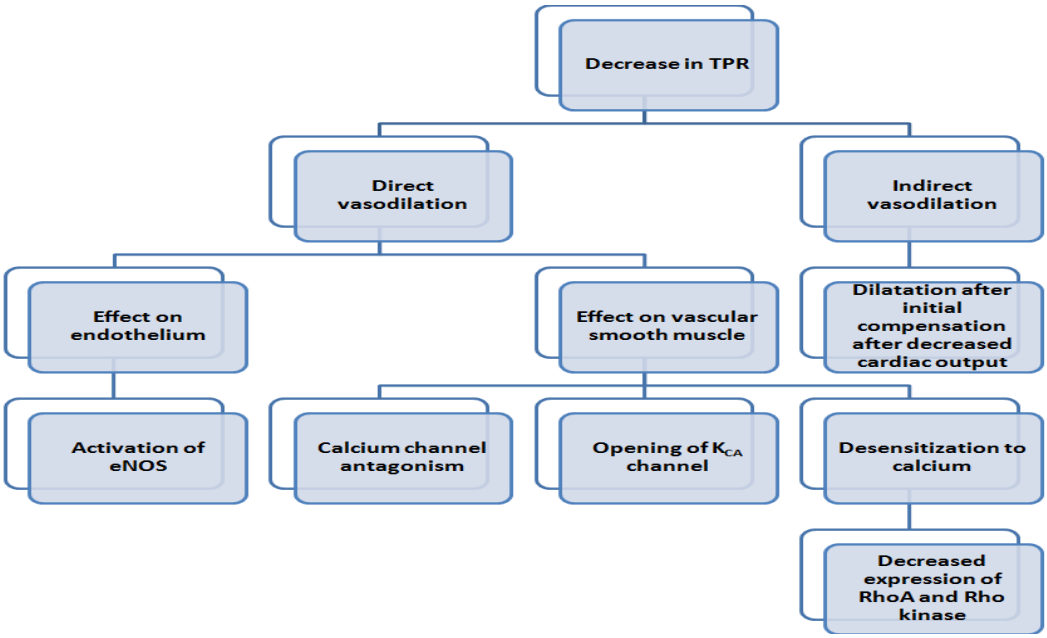


Fig.6: Theoretical hypothesis explaining thiazide's antihypertensive action on chronic use via modulation of total peripheral resistance

Hence to uncover the exact antihypertensive property of Thiazide class of drugs, a more comprehensive and multifaceted approach to hypertension research was needed and undertaken.

PHARMACOGENOMICS OF HYPERTENSION – CAN THIAZIDE RESPONSIVENESS BE GUIDED BY ONE'S GENES?

Genetic basis of Essential Hypertension susceptibility was estimated to be 30-70%. Uncovering of these gene foci could productively enhance the development of new chemical moieties for better care and benefit of the patient.

The genetic markers for salt sensitive forms of Essential Hypertension predilection, to name a few are: single nucleotide polymorphism (SNP) involving Angiotensin gene, alpha adducin (ADD1), the G-protein beta-3 subunit gene (GNB3) and the aldosterone synthase gene.[38]

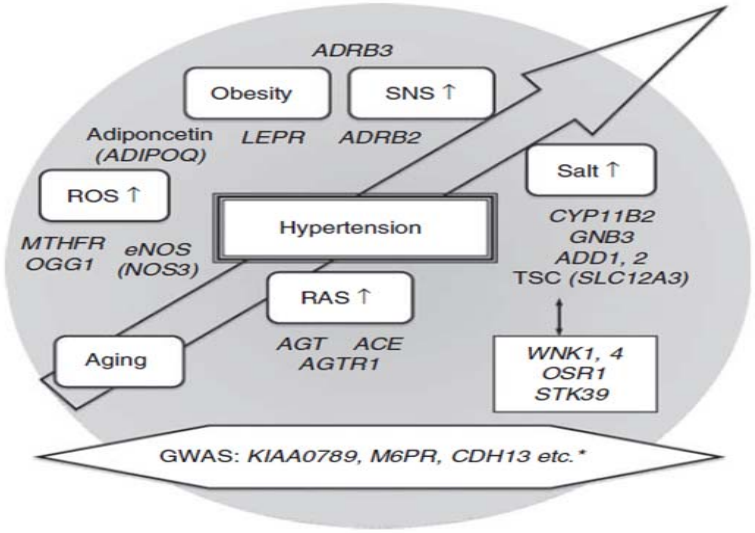


Fig.7: Genetic predictors of Hypertesion and potential pharmacogenetic targets of drug action.

The Thiazide sensitive NCC has a pivotal role in the treatment of hypertension, since Thiazide class of diuretics have been affirmed as the first line antihypertensive agent. The gene coding for this Na^+/Cl^- cotransporter (SLC12A3) was identified as a potential target in the antihypertensive agents pipeline.[39] Since this scientific fact was established, research to uncover the genetic basis of Thiazide action has been rigorously pursued.

Based on large scale Genome wide Linkage study, many candidate genes were uncovered as potential influencers of HCTZ responsiveness. Pharmacogenetic markers of HCTZ responsiveness that gained lot of research perusal are represented in the table below.

GENE NAME	GENETIC POLYMORPHISM IDENTIFIED	FUNCTION ALTERED
α-Adducin	ADD1_rs4963 ADD1A_rs4961	Increased renal tubular reabsorption of sodium ion through activation of Na/K-ATPase
Endothelial Nitric Oxide(NO) synthase	ENOSA_rs1799983	Regulates amount of NO in blood
G protein, β_3	GNB3_rs6489738	Mediates signal transduction across cell membranes
Sodium channel, gamma-subunit promotor	SCNN1G_rs5729 SCNN1G_rs5723 SCNN1G_rs7200183 SCNN1G_hcv11894753	Modulated transepithelial Na^+ transport
Sodium channel, nonvoltage-gated 1, beta	SCNN1B_rs250563	Modulates transepithelial Na^+ transport
Solute carrier family 12 Na^+/Cl^- transporters	SLC12A3_hcv9609124 SLC12A1_rs1552311	Regulation of renal sodium and chloride reabsorption

α-ADDUCIN GENE TARGET– HCTZ ANTIHYPERTENSIVE EFFICACY – POTENTIAL LEADS UNCOVERED:

Over the recent years, a genetic approach to understanding of the disease is on the rise. With development of phenotypic model of hypertension, namely the Spontaneous Hypertensive Rats (SHR) by Okamoto and Aoki in 1963 [40], the research to understand genetic predictors of disease and therapy has taken a fore step.

Experts opine that SHR models could be taken as counterpart for understanding clinical hypertension and its complications as well.[41] But with expansion of genetic tools , Genotypic models evolved to provide pin point focus on the genetic predictors of disease and disease progression.

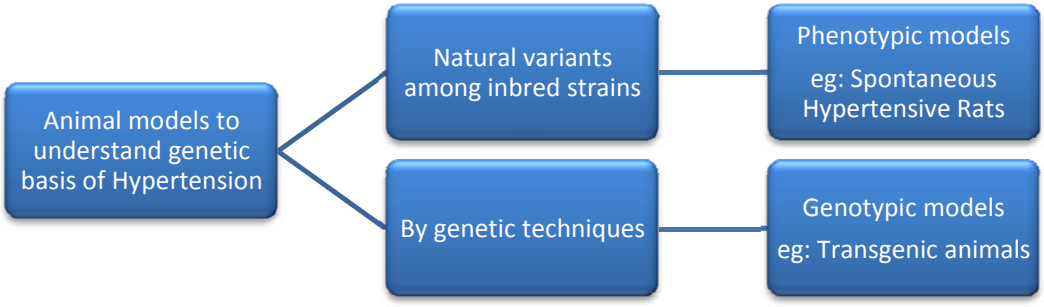


Fig.8: Genetic models for screening of antihypertensive activity in animals.

Milan Hypertensive strain (MHS) of rats were found do develop a form of hypertension with features of renal dysfunction analogous to changes seen in humans with Essential Hypertension. A point mutation in

the genes coding for adducing protein in MHS rats was implicated for this feature by Bianchi et al. in 1994. The MHS rat strains differed from their normal counterparts by the amino acid sequence at 316th position by replacement of tyrosine instead of phenylalanine. [42]

After 2 years of deducing this finding, Tripodi et al. tried to discover the molecular mechanism of this mutated variant of Adducin gene in MHS rats. Several renal cell lines of rat and human origin were genetically engineered to produce transfected wildtype or mutated ADD-1 genotypes. These modified genotypes showed increased expression and activity of Na^+/K^+ ATPase in the renal cells. Based on these observations, Torielli et. al postulated that mutated ADD-1 variants could be implicative in salt sensitive forms of Essential Hypertension.[43]

In 2004, further research on alpha Adducin by Efendiev et al. in MHS rats showed that the increased expression of Na^+K^+-ATPase did not undergo downexpression or endocytosis in response to dopamine stimuli. Hence the researchers concluded that the dynamic regulation to natriuretic stimuli could be impaired in alpha adducin mutants.[44]

Results from various research endeavours in this field, threw light on key points about Alpha Adducin. Adducin is a ubiquitously present tetrameric cytoskeletal protein made up of an α and either a β/γ heterodimeric subunits. It is coded by 3 genes ADD-1(α), ADD-2(β) and

ADD-3(γ). It is involved in inter-cellular contact, signal transduction and cell membrane ionic transport. By means of positional cloning, α Adducin has been mapped at chromosome 4p16.3

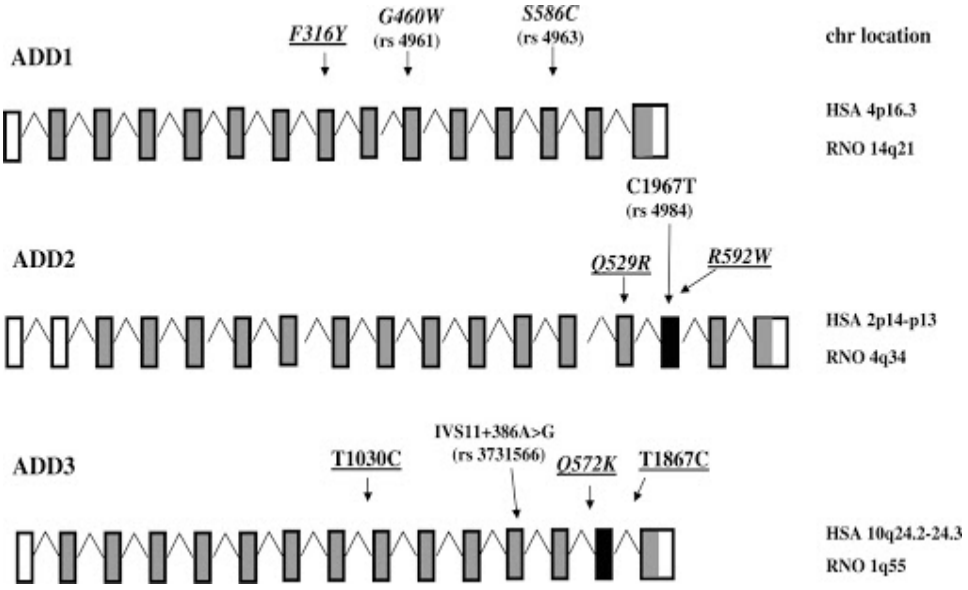


Fig.9: Mapping of Adducin loci in human genome.

Adducin is a term coined from Latin, 'adducere' meaning 'to bring together'. This cytoskeletal protein was initially identified as a component in the spectrin-actin framework of erythrocytes.[45]Subsequent studies identified its major role in promoting the organisation of spectrin-actin lattice formation. Adducin is a 200kD cytoskeletal protein belonging to the MARCKS- 'myristoylated alanine rich C kinase substrate' protein family.[46] It controls the rate of actin polymerization by capping the fast growing end of actin filaments. Its functioning is

dependent on calcium/calmodulin. It gets phosphorylated by Protein Kinase A/C, tyrosine and p-kinases.

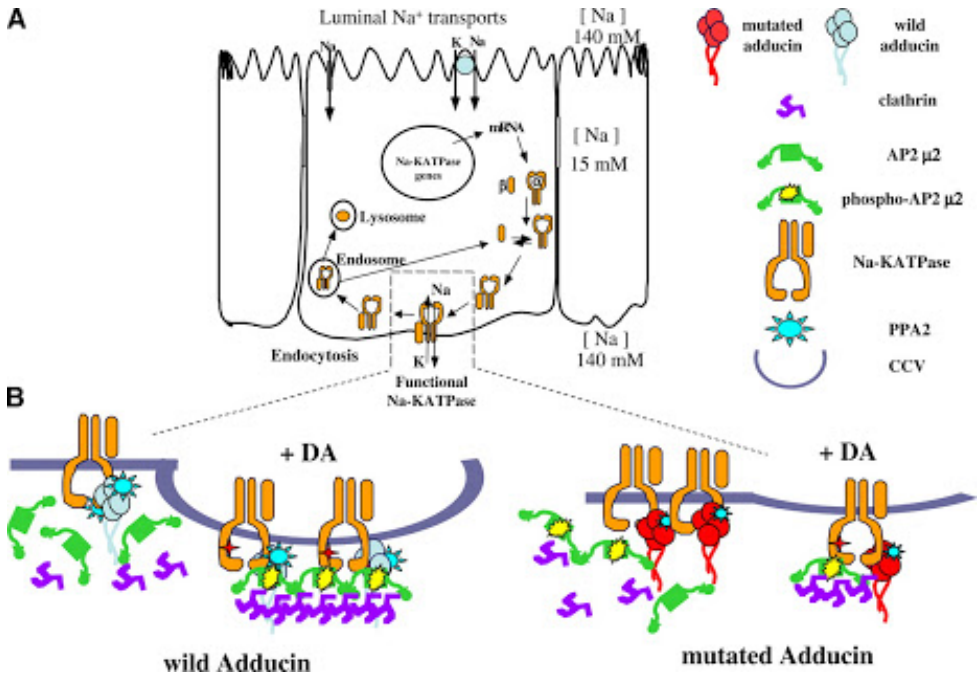


Fig.10: Schematic representation to differentiate mechanism of wild ADD-1 (Gly460Gly) and mutant ADD-1(Gly460Trp) on renal tubular Na/K-ATPase.

During the last decade of the 19th century, various researchers undertook genetic association studies to establish relationship of ADD-1 polymorphism to Essential Hypertension. In a case-control study conducted by Casari et al. in 1995, 190 Essential Hypertension and 126 Normotensive controls were screened for ADD-1 polymorphism linkage to Essential Hypertension. This study established a positive association.[47]

In 1997, genetic linkage of ADD-1 polymorphism, specifically Gly460Trp SNP variants were established to possess a strong linkage to salt sensitive forms of Essential Hypertension by Cusi et al.[48]Subsequently, research carried out by Manunta et al. on 108 Essential Hypertension patients confirmed that ADD-1 mutants with SNP 460Trp showed increased renal tubular sodium reabsorption.[49]

To further emphasise the relationship of Gly460Trp on renal tubular sodium reabsorption, Manunta et.al conducted endogeneous uric acid and lithium marker based studies to assess rate of renal tubular sodium reabsorption in Gly460Trp carriers. The results of their work established that Gly460Trp could be coined as 'renal hypertensive gene', since this variant was found strongly modulate renal sodium reabsorption and affect BP levels in Essential Hypertension subjects.[50]

The strength of relationship of Gly460Trp variant to Essential Hypertension was profound such that, the polymorphism was identified as an influential etiology for raised BP values in the genome-wide scan on cause of high BP values in familial combined hyperlipedemia among 18 Dutch families conducted by Allayee et al.(2001)[51]

In order to identify whether single nucleotide mutation in ADD-1/ADD-2/ADD-3 was responsible for high BP, Low oubain and renin activity, Lanzani et al.(2005) screened 512 newly detected

treatment naïve Essential Hypertension patients. The results of this study also favoured Gly460Trp linkage to salt sensitive Essential Hypertension.[52]

Translating this research finding into clinical utility the next escalatory approach of validation of Gly460Trp mediated salt sensitive Essential Hypertension. Hence researchers tried to investigate the responsiveness of Thiazide diuretics in Gly460Trp mutants.

Cusi et al. performed an interventional study on 37 Gly460Gly and 21Gly460Trp carriers of ADD-1 polymorphism, he treated these subjects with HCTZ 12.5mg for one month followed by 25mg dose the following month. The mean differences in MAP values between these two ADD-1 genotypes were assessed at the end of study duration. Their study results showed that Gly460Trp mutants had 7.9mmHg extra fall in MAP values than their homozygous counterparts receiving the same intervention.[53]

In the above study by Cusi et al., the genotype of the participants were already analysed prior to interventional drug administration. Hence subsequent research with the same intervention but post hoc genotyping was carried out by Glorioso et al. in 143 Caucasians (58 Italians, 85 sardinians). The results of this study also elucidated an added antihypertensive clinical efficacy of Gly460Trp over Gly460Gly Essential Hypertension patients. In addition, the study also showed ethnic

variation in response to HCTZ treatment between Italian and Sardinian subjects.[54]

Since ethnic variability in responsiveness of HCTZ existed within Caucasoid population themselves, many researchers independently took up this research question and tried to find answers among various countries and ethnicities.

In a Meta-Analysis conducted by Liu et al. in 2010, from pooling data from 22 studies researching α-Adducin polymorphism to Hypertension risk, the positive correlation of Gly460Trp to Essential Hypertension risk was seen predominantly in Caucasian, European and East Asian subgroups of population and negative association in Afro-American ethnicity.[55]

On further scrutiny on these positively proven associations, lot of discrepancies were reported. Among Caucasoid ethnic groups, population of Australian [56] and Polish [57] origins were not found to have significant association. Similarly among Asian ethnic groups, Han & Mongolian Chinese [58], Japanese [59] and Koreans [60] did not show significant linkage of ADD-1 460Trp and Essential Hypertension risk. Till date the role of ADD-1 460Trp among various ethnicities is still under speculation.

Focussing on Indian population and relationship of ADD-1 genotype mutant on Essential Hypertension risk, the studies reported have been scarce. Ramu et al., conducted a case control study among 432

Essential hypertensive patients and 461 healthy controls from South Indian population. Their results were inconclusive with regards to establishing association between ADD-1 460Trp and Essential Hypertension susceptibility.[61]

EXPERIMENTAL DRUGS USING ADDUCIN AS TARGET OF DRUG ACTION:

In spite of all the inconclusive evidence relating to ADD-1 460Trp-Essential Hypertension-Thiazide responsiveness, Researchers have identified a novel compound –'Rostafuroxin', a compound capable of modulating Adducin based Na/K-ATPase activity in the renal tubules and regulating Endogenous Oubain (A genetically mediated salt regulating hormone in the body) levels.

Rostafuroxin (PST2238)

This compound is a digitoxigenin derivative capable of selectively displacing Oubain from its binding site without affecting the other hormonal targets or endocrinal milieu involved in blood pressure regulation.[62]

In-vitro studies: Renal cells transfected with either ADD-1 460Trpmutation or Oubain exposure, when subjected to Rostafuroxin showed decrease in Na/K-ATPase activity, since the drug could counteract both oubain binding and alpha adducin mediated molecular mechanisms.[63]

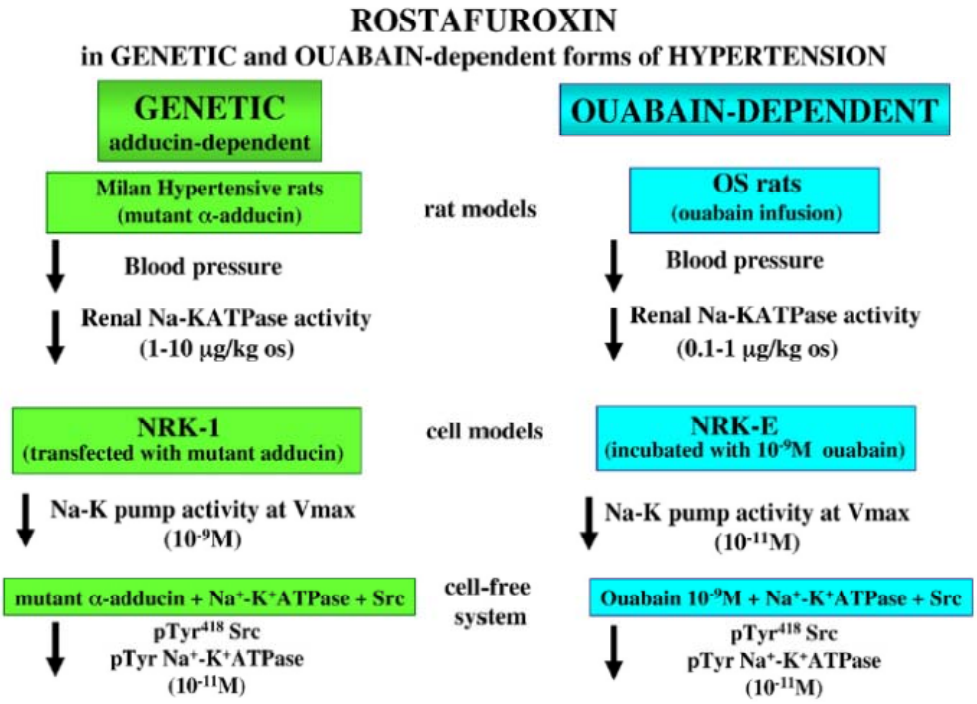


Fig.11: Rostafuroxin effects on Oubain and Alpha Adducin mediated Hypertensive changes in preclinical studies.

In-vivo animal studies:

Milan Hypertensive strains of rats have been taken as the effective animal model in studying effects of Rostafuroxin. The reason for selection of this strain is due to the fact that these MHS rats express ADD-1 polymorphism and increased Oubain levels. Both these genetic determinants are associated with salt sensitive Essential Hypertension and cardiac hypertrophy in MHS rats.[64]

MHS rats and Essential Hypertensive human subjects share many patho-physiological similarities with respect to Renal excretion of sodium after saline load, 24 hour urinary output, plasma renin, urine kallikrein as well.[65]

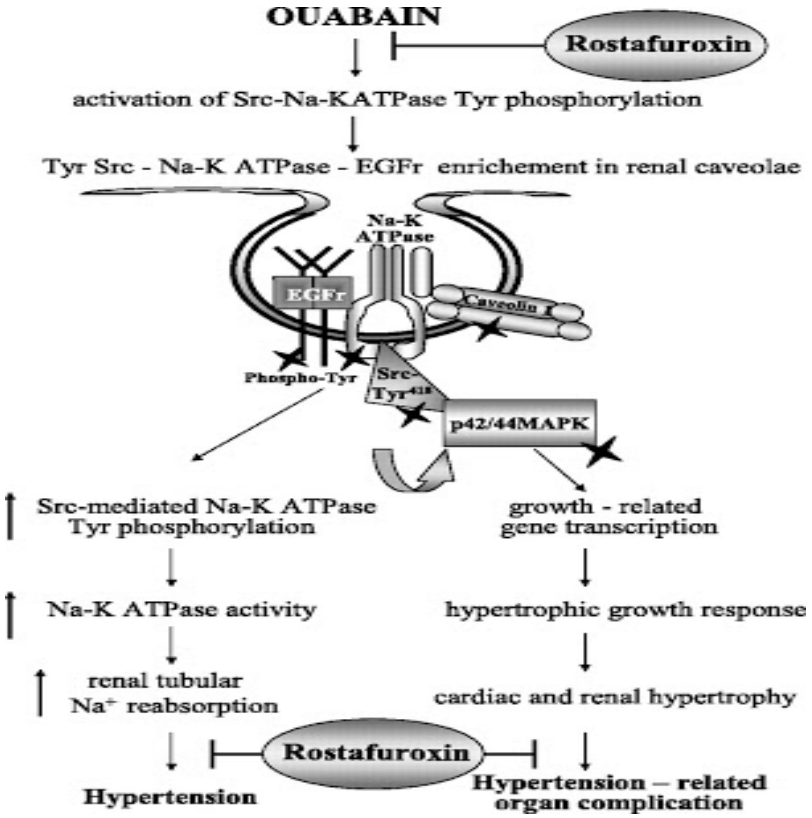


Fig.12: Molecular mechanism of action of Rostafuroxin

Results of Preclinical toxicity studies done in rats and monkeys demonstrated the following parameters[66]:

Acute toxicity following oral dose in rats	LD50 >2000mg/Kg
Chronic toxicity study (per os route) in rats	NOAEL = 250µmol/Kg/day
Chronic toxicity study (per os route) in Monkey	NOAEL = 450µmol/Kg/day
Genotoxicity	Nil

Rostafuroxin did not show inherent diuretic activity, hence the drug lacked diuretic-like adverse events such as alteration of uric acid/glucose/lipid metabolism, electrolyte abnormalities, counter-regulatory changes mediated by RAAS, etc.[67]

Clinical Trials with Rostafuroxin:

Rostafuroxin has successfully completed Phase I clinical trial in healthy volunteers.[67] Results from two exploratory Phase II studies done to establish efficacy and tolerability in mild Essential Hypertensive subjects was also successful and the effective dose was established as 0.1-1mg/day per oral.[67]

A multicentric Phase II Trial on 440 uncomplicated, moderate Essential hypertensive participants from Europian countries was undertaken to explore the impact of genetic variations in Adducin genotypes (ADD-1, ADD-2 and ADD-3) and enzymes involved in Oubain homeostasis on BP titration with Rostafuroxin.[68]Hence a pharmacogenetic strategy to tackle Essential hypertension is under processing and the results are imminent.

PHARMACOGENOMIC APPROACH TO MANAGEMENT OF HYPERTENSION – A COST-EEFECTIVE ANALYSIS:

Though Thiazide based diuretic combination with other classes of antihypertensive agents have been highly recommended as first step treatment, 80% of hypertensives do not take diuretics.[69] Most often when patients are started on a drug regime, the decision to switch over to a better agent happens only when the physician decides to revise the existent prescription or patient reports back to a health care facility for follow-up/complaints/morbidity.

The reason for revision or escalation of a monotherapy to combination regimes could be due to ineffectiveness or adverse reaction of the first prescribed agent in that individual patient.

Hence a rationale prescription to treatment of Essential hypertension should take into consideration about various factors affecting drug action in each individual patient from the initial stage of pharmacological intervention. One such individualised approach is utilization of the person's genomic profile and choosing a drug that would prove beneficial to the patient based on his/her genomic fingerprints.

Pre-prescription genotyping has been used as a tool for guiding use of some agents in routine practice. Examples are: CYP450 variants and VKORC1 variants in dosing serotonin reuptake inhibitors and warfarin

respectively.[70] Implementing a similar approach for guidance in prescription of medication for a complex polygenic disease like Essential hypertension in real time clinical setting could be quite daunting since Indian database of genomic evidence based medicinal practices are limited.[71]

Moreover for a complex polygenic disease, a single genotyping test alone would not provide a comprehensive list of all the genetic markers predicting drug action. But if that single gene plays a key role in dictating an individual's response to the effective drug available for treatment of the disease, then such a pharmaco-genotyping screening test could be a revolutionary and pharmaco-economic option as well.[72]

Meckley & Veenstra conducted a study questioning the pharmaco-economic outcome of using a pre-prescription screening test for Gly460Trp mutation among Essential Hypertensive for dictating usage of Thiazide diuretic over a conventional approach (no genotyping prior to drug administration). This study showed that patients who were screened for ADD-1 Gly460Trp mutation prior to Thiazide drug administration had an increased Quality Adjusted Life Years (QALYs) compared to their non screened counterparts. The genotypic screening saved $1834 compared to the conventional care as well.[73] This study also served to establish the influential role exerted by ADD-1 Gly460Trp on Thiazide effectiveness as an antihypertensive agent.

Hence an effective first step antihypertensive (Hydrochlorthiazide) with a determinant genetic target (ADD-1 Gly460Trp) translating to increased effectiveness of drug as well as pharmaco-economic benefit, with strong proof of evidence backed up by Rostafuroxin trials, with lacking evidence from Indian subset of population was a trigger to initiate our current study.

METHODOLOGY

This study was conducted in the Hypertension Out-patient clinic of Government Kilpauk Medical College during the period of June, 2014 to June, 2015. Out of the total 165 essential hypertensive patients screened, 100 subjects were included in the study, if the recruitment criteria were satisfied.

Study Design:

Allocation:	Stratified randomized
Endpoint Classification:	Safety/Efficacy Study
Intervention Model:	Parallel Assignment
Masking:	Open labelled
Primary Purpose:	Treatment
Condition :	Essential hypertension
Intervention :	T. Hydrochlorthiazide (HCTZ) 12.5mg once daily dose in the morning after breakfast

The study was conducted after the approval from the Institutional Ethics Committee, GKMC. Patients attending Hypertension clinic, Government Kilpauk Medical College were briefed and explained about the process and need for this current study. Patient information sheet and consent form were given to the patients. Following consent to participate

in the study, patients were subjected to baseline investigations and screening for eligibility to become a participant in the further proceedings.

Patients satisfying the following inclusion criteria were recruited in the study.

Inclusion Criteria:

1. Adult Essential Hypertensives (>18yrs, <70yrs) of either gender
2. Patients with Essential Hypertension on regular pharmacotherapy and atleast 3 consecutive monthly BP records entered in their OPD treatment books.
3. Essential hypertensives with associated co-morbidities: Renal dysfunction (Creatinine clearance > 60ml/min/sq.m), stable coronary artery disease, Old Cerebrovascular disease, controlled Diabetes mellitus

Exclusion Criteria:

1. Essential hypertensives (<18yrs, >70yrs), Pregnant women, Pregnancy related hypertensive disorders
2. Patients with known Secondary hypertension.
3. Essential hypertensive patients with poor glycemic control (HbA1c > 8.0%)

4. Patients with drug allergy to thiazide
5. Essential hypertensives with hepatic dysfunction, alcoholics, chronic heavy smokers
6. Hypertensives presenting with acute events or requiring In-patient care

Stratification factor :

Average of 3 consecutive monthly BP recordings. If values $\geq$ 140mmHg SBP or $\geq$ 90 mmHg DBP, patient was allocated in Intervention arm. Other patients with controlled BP recordings were assigned to the Control arm.

Baseline data collected on Day 1 and recorded in the case report form:

- Demographic details
- Anthropometry(BMI)
- Duration and course of disease with management details from medical records
- Blood Pressure readings from past 3 months
- Lab investigations: CBC, RFT, LFT, FLP, RBS

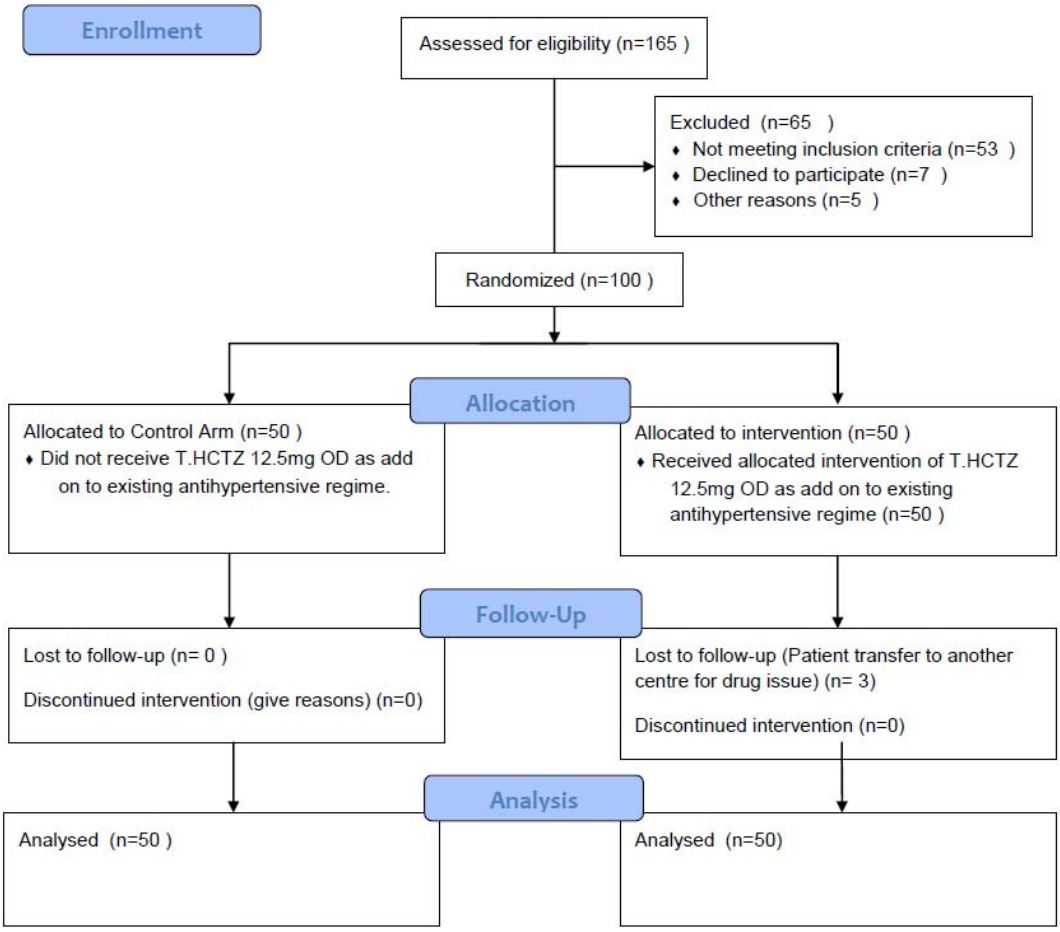


Fig.13: Participant Flow Diagram.
***Analysis of all 50 participants allocated to the Interventional arm was carried out by the last observation brought forward method.**

All patients were followed up on a monthly basis. On each visit, their weight and right arm sitting posture BP were monitored. Patients were questioned about their general wellness levels and specific side effects at each visit. 4th follow up visit (at the end of 3 months into study) included repeat of lab investigations.

A 2ml blood sample for genotyping of α-Adducin polymorphism was carried out during the first follow-up visit, when patient reports back with the results from baseline investigations. The recruitment of the patient on the interventional drug (HCTZ) group was determined irrespective of the Adducin status.

Sample collection:

Samples for baseline screening lab investigations were forwarded and processed by the Hospital Biochemistry wing. 2 ml of venous blood was collected under sterile aseptic precautions and transferred into EDTA tubes for genotyping procedures.

Procedure for Genotyping alpha – Adducin polymorphism:

Material & Methods:

Consumables for Genotyping - DNA purification kit (PureFast® Human Blood Genomic DNA purification kit), PCR Master Mix, Master Mix (2U of Taq DNA polymerase, 2mM MgCl2, 1µl of 10mM dNTPs mix, Taq reaction buffer and PCR additives), Agarose, TAE buffer, gel loading buffer and Ethidium bromide are from HELINI Biomolecules, Chennai.

Tetra ARMS PCR primers were also designed and synthesised by HELINI Biomolecules, Chennai.

Principle:

Cells are incubated for a short period with Proteinase K and chaotropic salts to inactivate all nucleases. Helini PureFast® kit utilizes an exclusive silica glass based membrane technology in the form of a convenient spin column, capable of recovering up to 20µg of DNA. Nucleic acids bound to this membrane is subjected to multiple "wash and spin" process for purification off cellular components. Final step uses low salt elution to release the nucleic acid from the glass fibre.

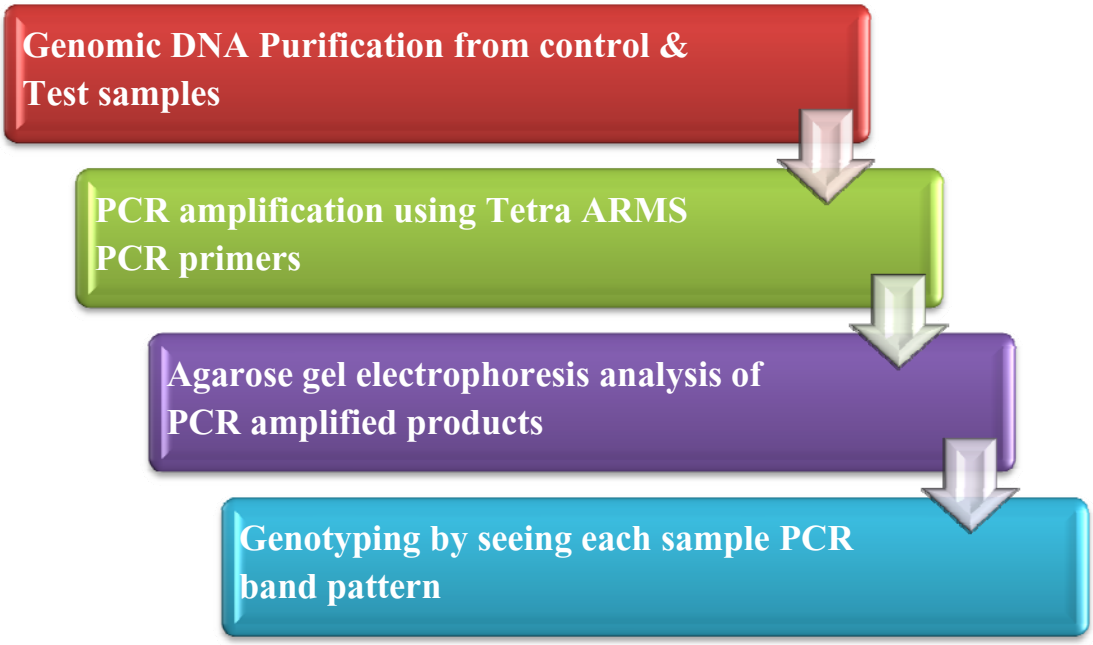


Fig.14: Overview of Genotyping procedure.

Procedure: Genomic DNA Extraction from venous blood sample

1. 200µl of EDTA treated venous blood sample is taken in a fresh 1.5ml centrifuge tube
2. 400µl of Lysis Buffer is added to the blood and gentle vortex mixing is done
3. To this mixture, 20µl of Proteinase K is added and vortex mixed for 10seconds
4. Incubated for 15 mins in a 56ºC hot water bath
5. Incubated sample is treated with 300µl of Isoproponal and entire sample is mixed well by inverting several times
6. The entire sample is transferred into the PureFast® spin column and centrifuged at 12000rpm for 1 min. The flow-through is discarded thereafter.
7. 500µl of 70% Ethanol is added into the spin column and centrifuged at 12000rpm for 1 min. Flow-Through is discarded
8. Step 7 is repeated again
9. Empty spin column centrifuge at 13000rpm for 2 mins is done to remove any residual alcohol from the sample
10. The membrane is transferred into a fresh labelled 1.5ml micro centrifuge tube
11. 60µl of pre-warmed Elution buffer is added to the centre of the membrane and incubated at room temperature for 2 mins
12. Final centrifuge for 1 min at 12000rpm is done and the purified DNA collected in the micro centrifuge tube is stored at -20ºC for subsequent processes

PCR Amplification using Tetra ARMS primers:

Primer for T allele:	5'-CCGGGGCGACGAAGCTTCCGAGGGAT-3' 5'- GATGTGGAGGTTCCTGCTAGTGTCACAGG-
Primer for G allele:	5'-GACTTGGGACTGCTTCCATTCTGTCC 5'GGACGAGAGAGACTGCAGCAAGGGTTT
PCR Product for T allele:	120bp
PCR product for G allele:	180bp
Two outer Primers:	248bp

Thermocycler PCR run was carried out with a Reaction Volume of 25µl (PCR Master Mix- 10µl, Primer-10µl and 5µl of DNA sample) in the following temperature settings: Initial denaturation at 95ºC – 5min, 35 cycles of (95ºC – 30sec, Annealing 60ºC – 30sec, 72ºC – 30sec) and final extension at 72ºC for 5 minutes.

Gel Electrophoresis:

2% agarose gel was prepared by mixing 0.6g of agarose powder with 30ml of Tris-Acetate-EDTA buffer and heating in a microwave for 1minute. 0.25µl of Ethidium bromide dye was mixed to this liquefied

agarose and poured onto the gel plate fixed with well cutter comb. After the gel sets, amplified PCR product was cast onto it. DNA ladder, Negative control and 6 PCR products could be loaded per electophoretic gel. Electrophoresis was carried out in a submarine type electrophoretic well apparatus at 100V for approximately 45 minutes. The gel film was then dismounted and used for band visualization immediately.

Visualization of DNA Bands:

The gel film is visualised under UV filter and camera capture of each run was saved in the computer software. Using the DNA ladder as a guide, DNA bands of Outer Primers (248bp), PCR product of T allele (120bp) and G allele (180bp) could be identified.

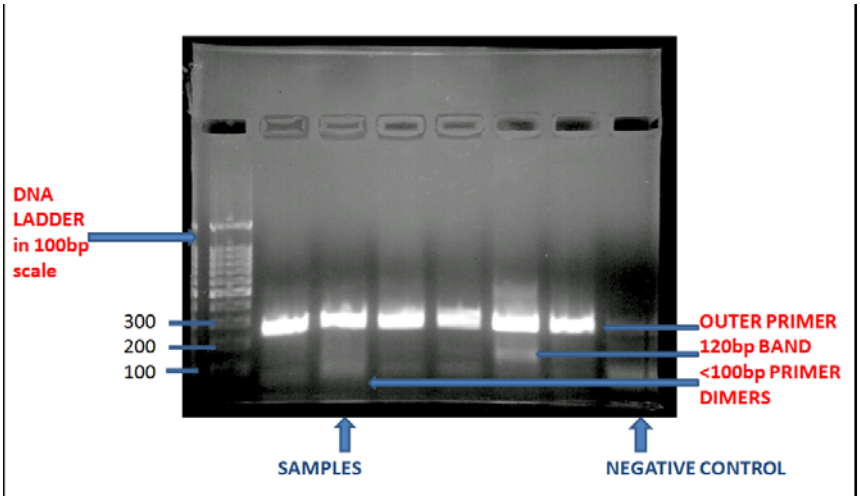


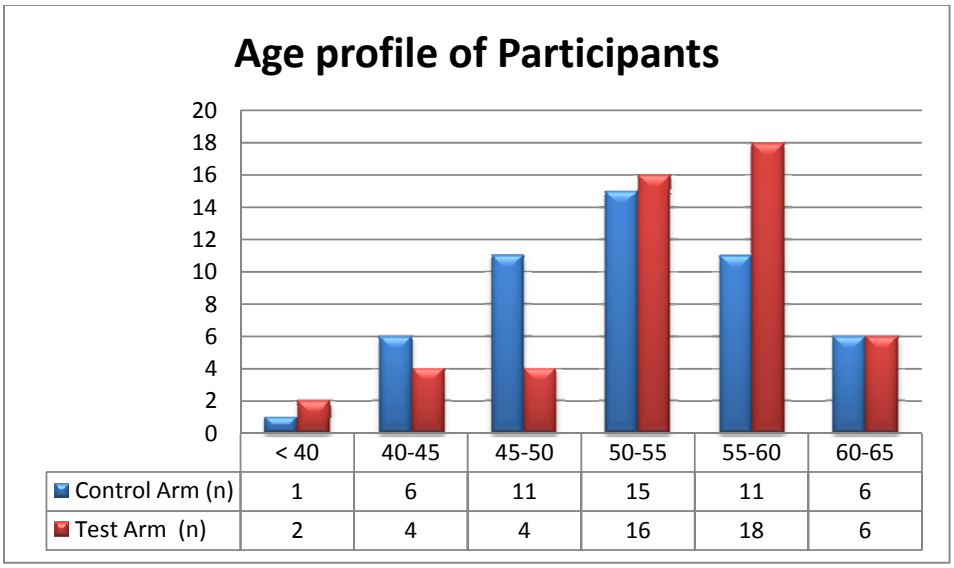
Fig.15: An example to explain interpretation of ARMS PCR gel electrophoresis film.

Statistical Analysis:

All patient particulars were charted onto Microsoft 2007 Excel sheet systematically. Descriptive statistics was applied to describe demographic and laboratory investigations. Microsoft Excel generated graphical representations of data were also included to express results. SPSS software version 13.0 was used. Inferential statistics was applied to assess difference in means between and within groups of allocation and genotypes (ANOVA, t test). Chi square test was used to determine statistical significance and establishing association between each subset projected onto the entire group. Levene's Test for Equality of Variances to assess impact of HCTZ among Genotypically classified Control and Test arms was also assessed.

RESULTS

1. Demographic characteristics of Study Population:

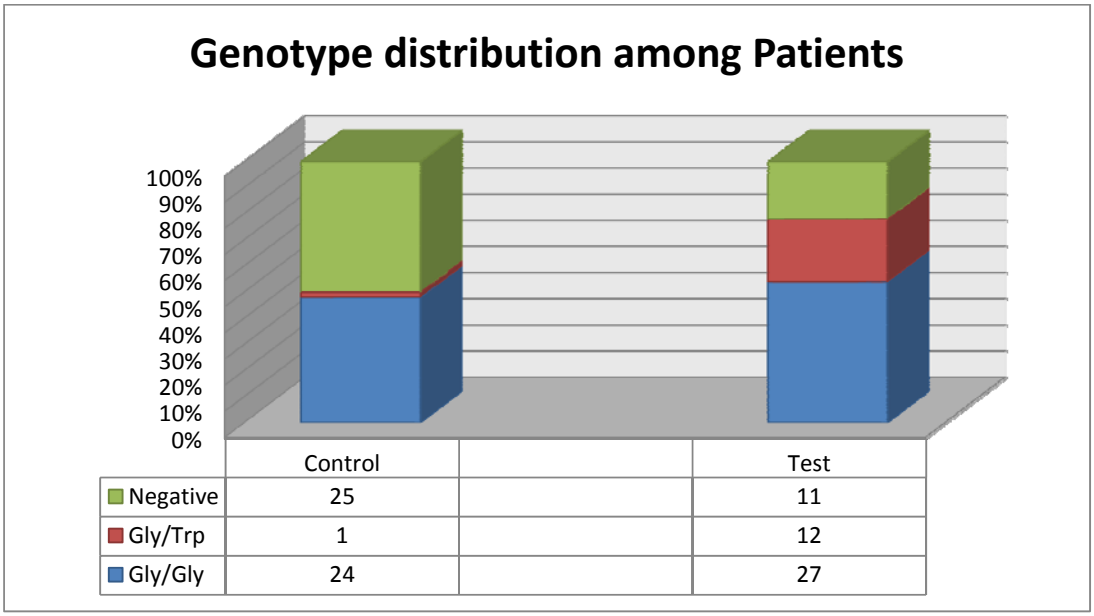


	< 40	40-45	45-50	50-55	55-60	60-65
Control Arm (n)	1	6	11	15	11	6
Test Arm (n)	2	4	4	16	18	6

Age of study participants ranged from 36 to 64 years. The mean age of study participants was 52.16years (Average age of Control and Test arm participants was 51.58yrs and 52.74yrs respectively.)

The study participants fell under three ADD-1 Genotype categories when ARMS-PCR assay was carried out. The Genotype categories were: Gly/Gly, Gly/Trp, Negative. The primary study objective was to assess the relationship of Gly/Trp to Essential Hypertension and Hydrochlorthiazide responsiveness.

Genotype based distribution of ADD-1 gene among the 100 Essential Hypertension patients allocated to Test and Control arms is represented by the graph that follows:



Age wise Genotype distribution among recruited subjects:

			Genotype			Total	P value
			Gly/Gly	Gly/Trp	Negative		
Age in years	18-40	Count	2	1	2	5	0.489
		% within Age in years	40.0%	20.0%	40.0%	100.0%	
		% within Genotype	3.9%	7.7%	5.6%	5.0%	
	41-50	Count	20	2	8	30	
		% within Age in years	66.7%	6.7%	26.7%	100.0%	
		% within Genotype	39.2%	15.4%	22.2%	30.0%	
	51-60	Count	27	10	24	61	
		% within Age in years	44.3%	16.4%	39.3%	100.0%	
		% within Genotype	52.9%	76.9%	66.7%	61.0%	

	Above 60	Count	2	0	2	4	
		% within Age in years	50.0%	.0%	50.0%	100.0%	
		% within Genotype	3.9%	.0%	5.6%	4.0%	
Total		Count	51	13	36	100	
		% within Age in years	51.0%	13.0%	36.0%	100.0%	
		% within Genotype	100.0 %	100.0%	100.0%	100.0%	

Gender wise Genotype distribution among patients:

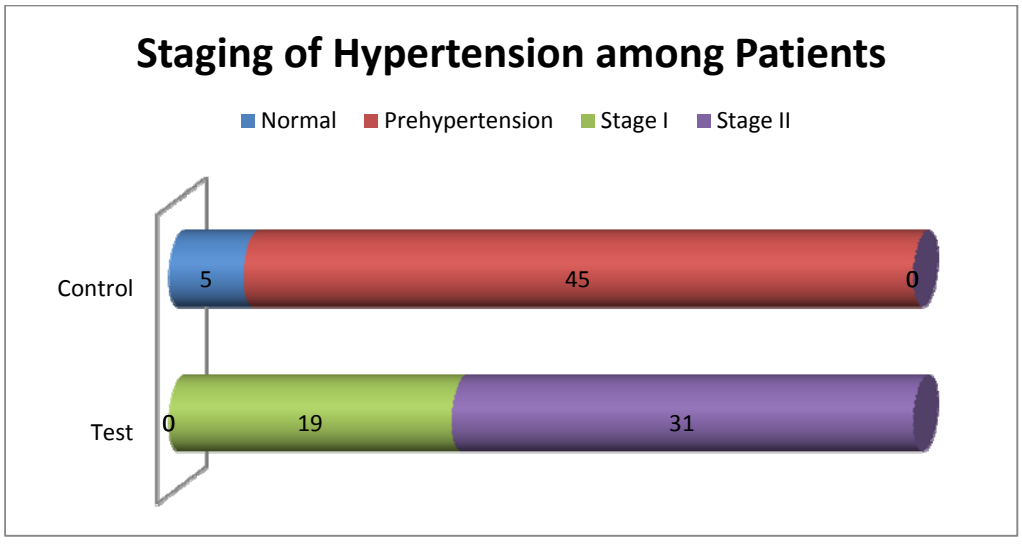
			Genotype			**Total**	**Chi square P value**
			Gly/Gly	Gly/Trp	Negative		
Gender	Male	Count	23	9	18	50	.299
		% within Gender	46.0%	18.0%	36.0%	100.0 %	
		% within Genotype	45.1%	69.2%	50.0%	50.0%	
	Female	Count	28	4	18	50	
		% within Gender	56.0%	8.0%	36.0%	100.0 %	
		% within Genotype	54.9%	30.8%	50.0%	50.0%	
Total		Count	51	13	36	100	
		% within Gender	51.0%	13.0%	36.0%	100.0 %	
		% within Genotype	100.0%	100.0 %	100.0%	100.0 %	

Age and gender based distribution of α-Adducin genotype among the participants did not show any statistical significance as seen in the crosstables above.

2. Hypertension profile of study participants:

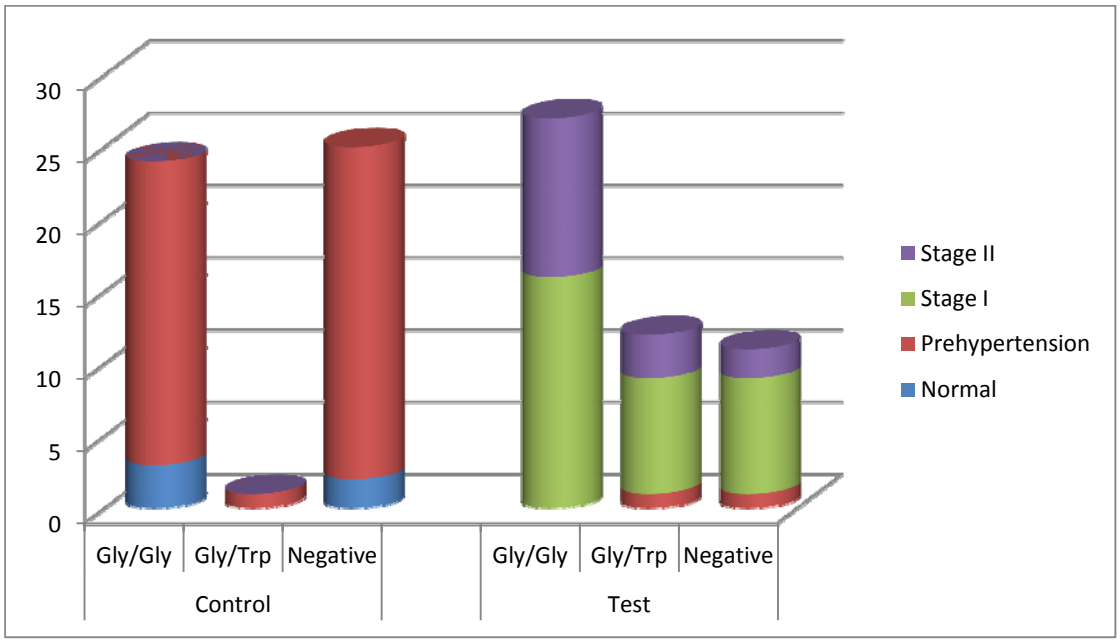
As per the study protocol, Patients were recruited into Test and Control arms based on the SBP/DBP cut-offs. Average three monthly records that were consistently > 140/90mmHg (in spite of pharmacotherapy) was taken as the stratification set point. Hence among Control arm, all the participants fell in Normal or Prehypertension grade (with pharmacotherapy) of JNC- 8 grade of hypertension. Similarly, Test participants fell under Stages I & II only.

Stage of Hypertension among Control and Test Participants



In the Control arm, 5 patients had Normal BP and 45 patients fell in the Pre-Hypertension stage. The Test arm included 19 patients in Stage I and 31 patients in Stage II Hypertension respectively.

Genotype of participants with regards to their baseline grading of Hypertension:



			Genotype			Total	P value
			Gly/Gly	Gly/Trp	Negative		
Staging (Baseline)	Normal	Count	3	0	2	5	
		% within Staging	60.0%	.0%	40.0%	100.0%	
		% within Genotype	5.9%	.0%	5.6%	5.0%	.018*
	Pre HT	Count	21	2	24	47	
		% within	44.7%	4.3%	51.1%	100.0%	

		Staging					
		% within Genotype	41.2%	15.4%	66.7%	47.0%	
	Stage 1	Count	16	8	8	32	
		% within Staging	50.0%	25.0%	25.0%	100.0%	
		% within Genotype	31.4%	61.5%	22.2%	32.0%	
	Stage 2	Count	11	3	2	16	
		% within Staging	68.8%	18.8%	12.5%	100.0%	
		% within Genotype	21.6%	23.1%	5.6%	16.0%	
Total		Count	51	13	36	100	
		% within Staging	51.0%	13.0%	36.0%	100.0%	
		% within Genotype	100.0%	100.0%	100.0%	100.0%	

Among the 100 study participants, 51% had Gly460Gly and 13% had Gly460Trrp genotype of α-Adducin. 36% of patients were negative for either of the genotypic traits. Baseline staging wise genotype distribution showed a statistical significance (p-0.018)

Descriptives of Baseline Mean Arterial Pressure based on Genotype:

	N	Mean	Std. Deviation	95% Confidence Interval for Mean		Min value	Max value	P value
				Lower Bound	Upper Bound			0.012
Gly/Gly	51	105.2941	11.17092	102.1522	108.4360	83.33	126.67	
Gly/Trp	13	109.5385	6.84422	105.4025	113.6744	96.67	120.00	
Negative	36	100.3889	9.66010	97.1204	103.6574	83.33	120.00	
Total	100	104.0800	10.55084	101.9865	106.1735	83.33	126.67	

Mean Arterial Pressure (Calculated) values, when sorted according to the genotypes, also showed statistically significant intergroup differences when Chi-square test was applied.

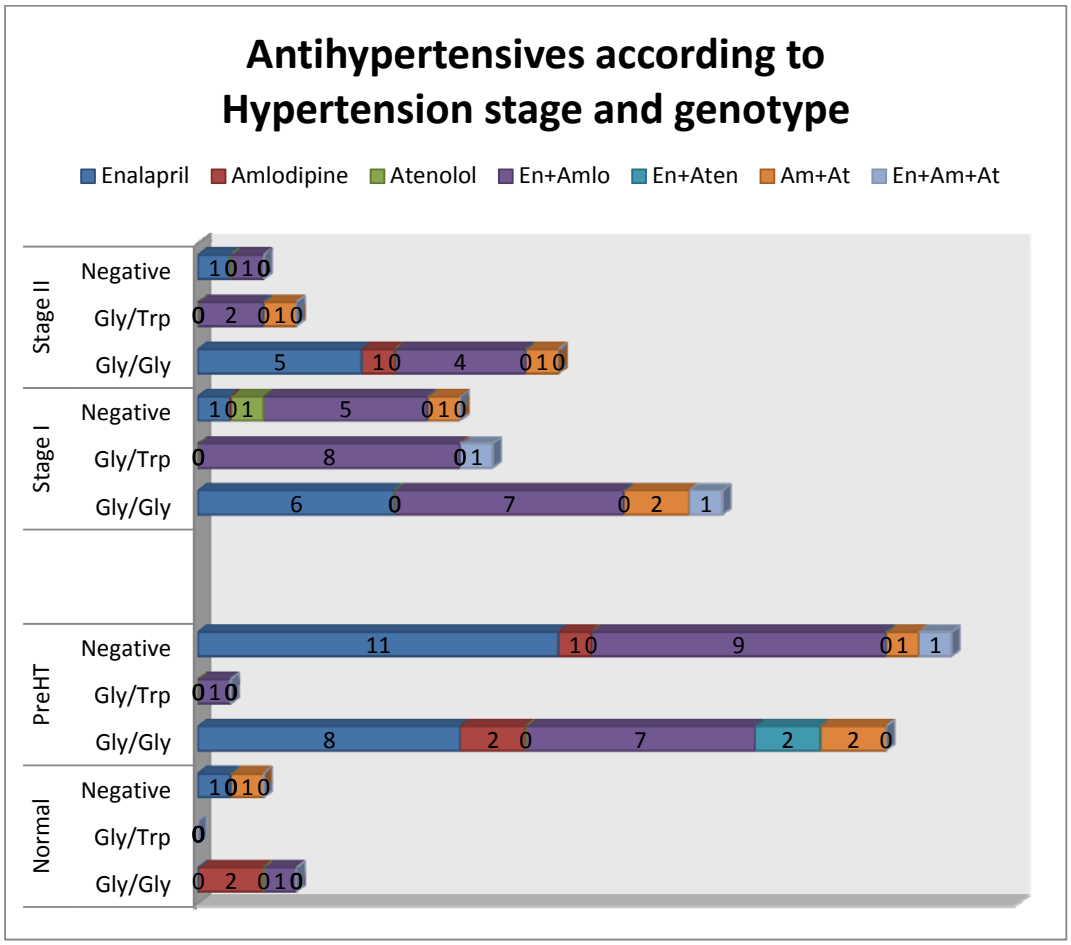
3. Baseline Antihypertensive agents used among Control and Test arm

Three classes of pharmacological intervention were being used in our site.

Namely :

- ACE inhibitor – T. Enalapril 2.5mg
- Calcium Channel Blocker-T.Amlodipine 2.5mg
- Cardioselective Beta Blocker – T. Atenolol 50mg

Among the Control Arm, the most common antihypertensive used was Enalapril monotherapy (especially in Prehypertension stage), other participants most often had a combination of Amlodipine plus various other classes of antihypertensives available.



The Test arm participants were most often treated with Enalapril based combination with either Amlodipine/Atenolol or all three agents together. In spite of using all the three classes of antihypertensive agents in our setup, their average consecutive three monthly BP values remained above 140/90mmHg.

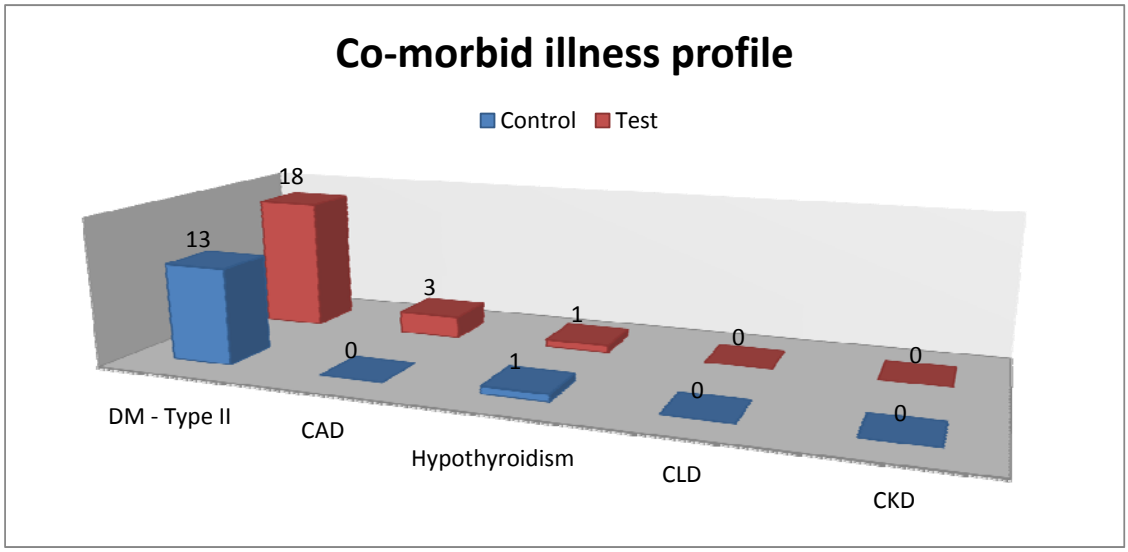
In our study, patients with a consecutive three monthly average BP over 140/90mmHg in spite of using 2 or 3 different classes of pharmacological agents (though none of the drug regimen included a diuretic) were recruited in the Test arm and received T.HCTZ 12.5mg

once in the morning dose for 3 months along with their baseline antihypertensives.

4. Co-morbid Illness profile

Co-morbid illnesses seen among recruited EH patients were: Diabetes Mellitus- Type II, Coronary Artery Disease, Hypothyroidism.

Medical records of management of Co-morbid illnesses were traced and relevant treatment history was also taken into account.

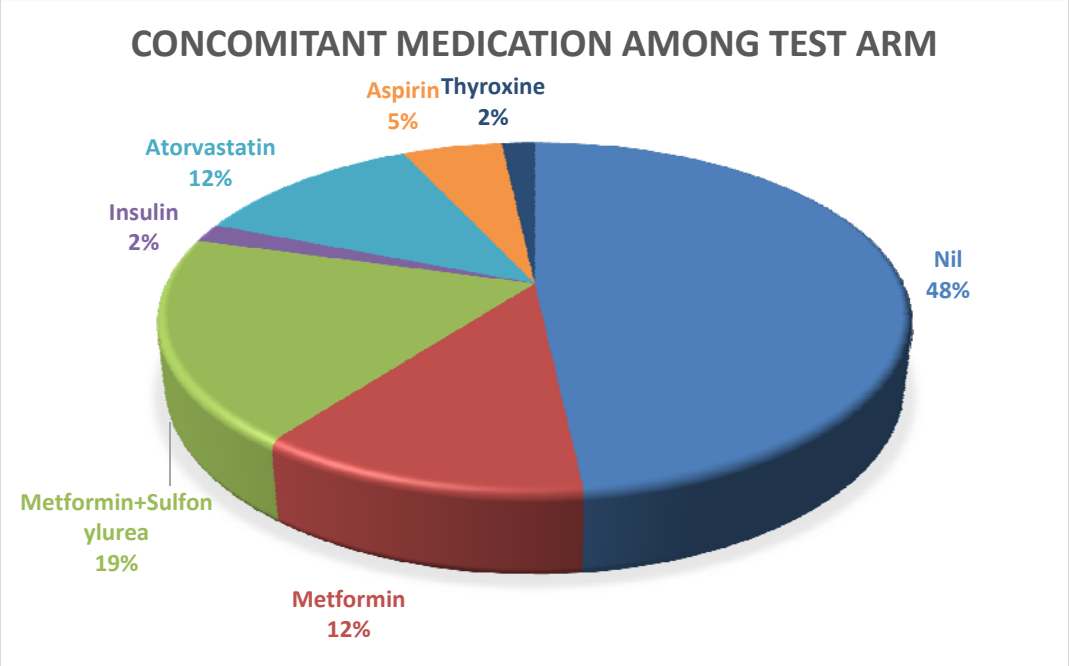


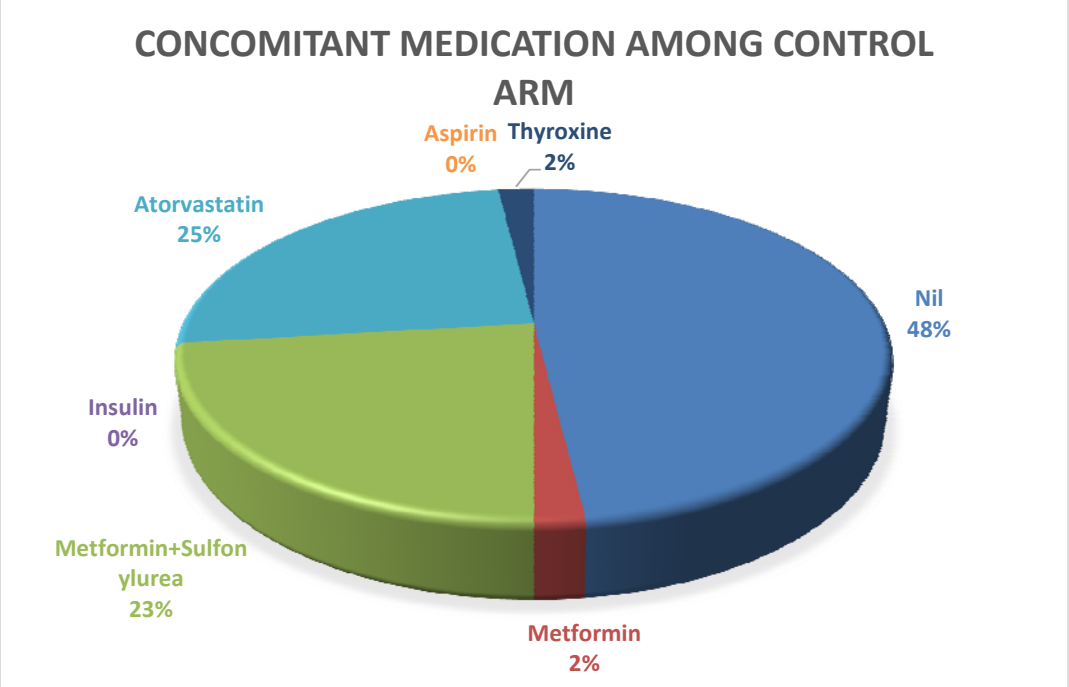
As per the study protocol, patients with documented Chronic Liver/Kidney diseases were not included in study.

5. Concomitant Medication usage among Participants

50% of participants in the Control arm and 56% of participants in the Test arm did not receive any other concomitant medicaments. The rest of

the study participants were receiving treatment for their Diabetes and Coronary Artery Disease. Both the Test and Control arm had one EH patient with hypothyroidism on Thyroxine supplementation.





6. Laboratory parameter profile of Participants

	N	Range	Minimum	Maximum	Mean
Baseline_HB	100	5.80	9.20	15.00	12.1280
Baseline_platelet	100	203000.00	148000.00	351000.00	236033.0000
Baseline_Bilirubin	100	.40	.60	1.00	.8060
Baseline_protein	100	2.40	5.60	8.00	6.9320
Baseline_SGOT	100	24.00	16.00	40.00	29.7700
Baseline_SGPT	100	44.00	10.00	54.00	25.8800
Baseline_SAP	100	56.00	44.00	100.00	66.3700
Baseline_Urea	100	20.00	16.00	36.00	27.2600
Baseline_Creatine	100	.70	.60	1.30	.8370
Baseline_Sodium	100	30.00	114.00	144.00	137.0700
Baseline_Pottasium	100	2.00	3.00	5.00	3.8730
Baseline_RBS	100	264.00	70.00	334.00	145.5000
Baseline_Cholesterol	100	187.00	92.00	279.00	171.7500
Baseline_Triglycerides	100	285.00	75.00	360.00	157.9900
Valid N (listwise)	100				

The above table shows the Range, Average, Maximum and Minimum values of all the baseline screening laboratory investigations taken from all the 100 subjects.

7. Hydrochlorthiazide intervention versus Genotype distribution

			Genotype			**Total**	**Chi square p value**
			Gly/Gly	Gly/Trp	Negative		
HCTZ	No	Count	24	1	25	50	.001
		% within HCTZ	48.0%	2.0%	50.0%	100.0%	
		% within Genotype	47.1%	7.7%	69.4%	50.0%	
	Yes	Count	27	12	11	50	
		% within HCTZ	54.0%	24.0%	22.0%	100.0%	
		% within Genotype	52.9%	92.3%	30.6%	50.0%	
Total		Count	51	13	36	100	
		% within HCTZ	51.0%	13.0%	36.0%	100.0%	
		% within Genotype	100.0%	100.0%	100.0%	100.0%	

On Statistical evaluation to assess relationship between Hydrochlorthiazide administration and genotype profile(Gly460Gly/ Gly460Trp / Negative) among test and control arm, the results were highly significant.

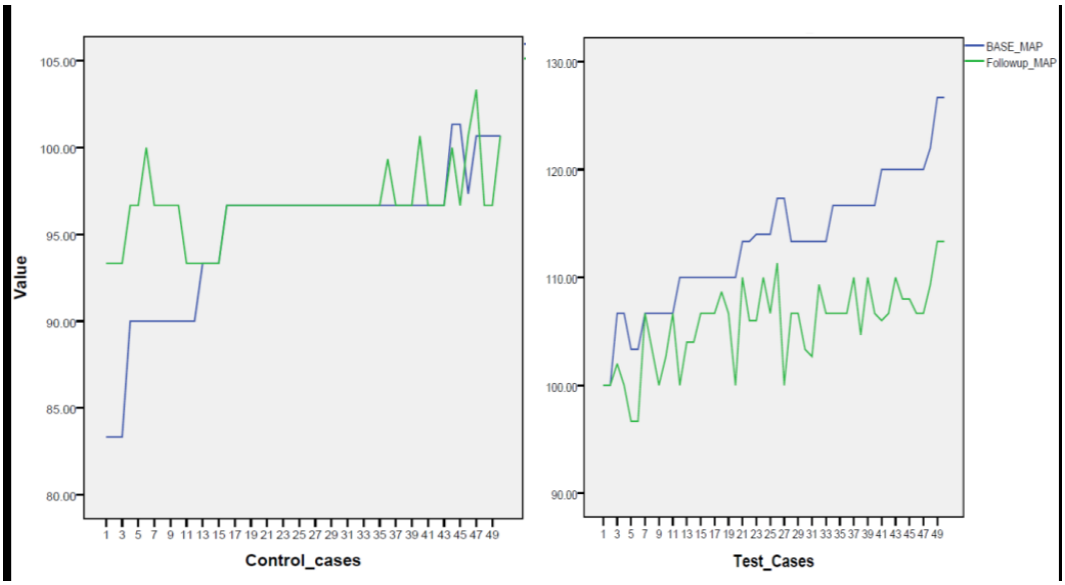
8. Follow-up vs Baseline Blood Pressure changes among test and control arm

Decrement in BP(Follow-up minus Baseline values)	Allocation	N	Mean	Std. Deviation	Std. Error Mean
SBP - Difference	Control	50	-1.3600	3.66873	.51884
	Test	50	-9.2000	7.22806	1.02220
DBP - Difference	Control	50	-1.8800	4.50687	.63737
	Test	50	-6.6800	4.47414	.63274
MAP - Difference	Control	50	-1.7067	3.61443	.51116
	Test	50	-7.5200	4.24219	.59994

Mean Arterial Pressure is a calculated parameter.

[MAP = DBP+1/3(SBP-DBP)]

Hence a MAP value above 106.67mmHg could be considered as allocation into Test arm of study. A schematic representation of the change in MAP values after three months of conventional pharmacotherapy (among control subjects) and additional HCTZ therapy (among test subjects) is as follows:



Patients who received Hydrochlorthiazide 12.5mg as an addition to their existent antihypertensive agents, invariably had a reduction in their SBP, DBP and calculated MAP values from their corresponding baseline recordings.

9. Genotype influence on BP changes among Test and Control

When all 100 participants were assessed based on their genotype status to assess for intergroup variation in changes in MAP values, statistical significance was established. (Chi-square test)

	N	Mean	Std. Deviation	95% Confidence Interval for Mean		Min value	Max value	P value
				Lower Bound	Upper Bound			0.019
Gly/Gly	51	101.93	5.712	100.33	103.54	93	113	
Gly/Trp	13	103.49	4.862	100.55	106.43	97	110	
Negative	36	99.26	4.825	97.63	100.89	93	110	
Total	100	101.17	5.469	100.09	102.26	93	113	

Subsequently, Levene's Test for Equality of Variances to assess impact of HCTZ among Genotypically classified Control and Test arms was applied. The following results showed highly significant statistical P values.

		F	Sig.	t	df	Sig. (2-tailed)
SBP - Difference	Equal variances assumed	22.134	.000	-9.212	98	.000
	Equal variances not assumed			-9.212	72.676	
DBP - Difference	Equal variances assumed	.629	.430	-9.531	98	.000
	Equal variances not assumed			-9.531	97.995	
MAP - Difference	Equal variances assumed	1.872	.174	-11.706	98	.000
	Equal variances not assumed			-11.706	95.590	

The results of ANOVA parametric test to establish statistical significance to test the difference in means of SBP, DBP and MAP values and the influence of ADD-1 Genotypes (Gly460Gly ,Gly460Trp and Negative) on BP reduction among the Test arm of patients are follows:

		Sum of Squares	df	Mean Square	F	Sig.
SBP - Difference	Between Groups	475.652	2	237.826	4.170	.018
	Within Groups	5531.708	97	57.028		
	Total	6007.360	99			
DBP - Difference	Between Groups	209.151	2	104.575	2.819	.065
	Within Groups	3598.849	97	37.102		
	Total	3808.000	99			
MAP - Difference	Between Groups	252.692	2	126.346	3.607	.031
	Within Groups	3397.548	97	35.026		
	Total	3650.240	99			

When individual genotype influence on HCTZ responsiveness in the 50 test population was assessed, Gly460Gly and Negative carriers had significant results. Gly460Gly homozygous genotype was seen in 27(54%) test participants.

The mean reduction in their SBP, DBP and MAP values following HCTZ add on for 3 months are as follows: 11.41, 6.07, 7.85 mmHg respectively. 11(22%) test subjects were negative for ADD-1 polymorphism, their mean decline in BP parameters after HCTZ use was SBP by 6.18, DBP by 8.55 and MAP by 7.76mmHg. These BP decrements were also significant with respect to genotype variation in BP reduction with HCTZ usage.

Since Analysis of Variance results were significantly different among the three genotypic groups, individually each genotype based comparison was done to assess their influence on decrements in BP values among Test and Control arms were assessed.

- **Genotype Gly/Gly**

	Allocation			
	Control		**Test**	
	Mean	**SD**	**Mean**	**SD**
SBP - Difference	-1.58	4.13	-11.41	6.32
DBP - Difference	-1.75	4.22	-6.07	4.35
MAP - Difference	-1.69	3.26	-7.85	4.37

Levene's Test for Equality of Variances

		F	**Sig.**	**T**	**df**	**Sig. (2-tailed)**
SBP - Difference	Equal variances assumed	4.116	.048	-8.571	49	<.001
	Equal variances not assumed			-8.780	45.179	
DBP - Difference	Equal variances assumed	1.095	.300	-6.499	49	<.001
	Equal variances not assumed			-6.510	48.596	
MAP - Difference	Equal variances assumed	5.480	.023	-8.752	49	<.001
	Equal variances not assumed			-8.903	47.642	

Among the Gly460Gly participants, between the Test and Control arms, statistical high significance was established as an impact of using HCTZ in test subjects.

- **Genotype Gly/Trp**

	Allocation			
	Control		Test	
	Mean	SD	Mean	SD
SBP - Difference	.00	.	7.00	7.56
DBP - Difference	.00	.	6.33	5.84
MAP - Difference	.00	.	6.56	5.00

Since there was only one Gly460Trp participant in the control arm and 12 in the Test arm, Comparability and applicability of inferential statistical tests were obsolete.

- **Genotype Negative**

	Allocation				**Levene's Test**
	Control		**Test**		**For equality of variance**
	Mean	**SD**	**Mean**	**SD**	**Sig. (2-tailed) p value**
SBP - Difference	-1.20	3.32	-6.18	7.77	<.001
DBP - Difference	-2.08	4.92	-8.55	2.54	<.001
MAP - Difference	-1.79	4.04	-7.76	3.09	<.001

Between the test and control participants with neither ADD-1genotypic variants, statistically high significant decrement in BP was seen as a result of adding adjuvant HCTZ to the test arm. This result was similar to Gly/Gly Variant.

10. Influence of Gly/Trp on BP changes vs other Genotypes (Gly/Gly and Negative) in Test arm

ANOVA was applied to assess inter-genotypic variation in delta MAP values (Follow-up minus Baseline MAP) in the 50 patients allotted in Test arm alone.

Groups	*Count*	*Sum*	*Average*	*Variance*	**P value**
Gly/Gly	27	-212	-7.85185	19.08832	0.672622
Gly/Trp	12	-78.6667	-6.55556	25.03704	
Negative	11	-85.3333	-7.75758	9.535354	

On individually comparing Gly/Trp group vs Gly/Gly and Negative genotypes, the following results were obtained.

Gly/Trp vs Negative

Groups	*Count*	*Sum*	*Average*	*Variance*	*P-value*
Gly/Trp	12	-78.6667	-6.55556	25.03704	0.500634
Negative	11	-85.3333	-7.75758	9.535354	

Gly/Trp vs Gly/Gly

Groups	*Count*	*Sum*	*Average*	*Variance*	*P-value*
Gly/Trp	12	-78.6667	-6.55556	25.03704	0.418523
Gly/Gly	27	-212	-7.85185	19.08832	

Though there was a statistically high significant inter-genotypic difference with regards to BP changes between Control and Test arm subjects, when ANOVA was applied to assess the same question in Test arm subjects alone, results were not statistically significant. Meaning, that Gly460Trp carriers did not show superior HCTZ antihypertensive response than Gly40Gly or Negative genotype carriers. Since ANOVA parametric test to assess difference in mean of MAP changes between the genotype classes did not produce significant results, t test was not required further.

11. Safety check on HCTZ use among test subjects.

Parameter	Control	Test
Follow-up Hemoglobin (g/dl)	12.18+1.11	12.17+1.12
Follow-up Platelets (cells/cu.mm)	235886+50098.38	235589.58+50105.15
Follow-up Total Protein (g/dl)	6.90+0.39	6.90+0.39
Follow-up Total Bilirubin (mg/dl)	0.81+0.08	0.81+0.08
Follow-up SGOT (U/L)	29.63+6.17	29.53+6.23
Follow-up SGPT (U/L)	26.07+8.41	26.16+8.53
Follow-up SAP (IU/L)	66.37+14.19	66.53+14.29
Follow-up Urea(mg/dl)	27.26+4.55	27.44+4.53
Follow-up Creatinine (mg/dl)	0.837+0.13	0.84+0.14
Follow-up Sodium (mEq/L)	137.48+3.83	137.49+3.86
Follow-up Potassium (mEq/L)	3.80+0.32	3.79+0.33
Follow-up Random Blood Sugar (mg/dl)	147.45+48.24	149.47+48.11
Follow-up Total Cholesterol (mg/dl)	172.73+39.07	172.36+39.67
Follow-up Triglycerides (mg/dl)	156.40+61.17	157.11+61.91

Hydrochlorthiazide 12.5mg once daily dosage, as an add- on agent in management of Essential hypertension in the 50 Test participants did not produce significant adverse events. The follow-up laboratory parameters did not show significant variation from the counterpart recordings among the Control arm participants.

DISCUSSION

This current study was conducted among 100 Essential Hypertension patients to find the prevalence of α-Adducin mutation (Gly460Trp) and assess association of this genotypic variant on Blood Pressure control obtained by Hydrochlorthiazide add-on therapy among South Indian population.

Equal gender distribution was made possible by means of effective block randomization techniques used during recruitment of study participants. The mean age of study participants was 52.16years, with male and female gender wise age averages being 52.48 and 51.84 years respectively. Hence age and gender based variability of study parameters were not necessitated in the subsequent analysis.

The genotype distribution among study participants fell under three categories: Gly460Gly, Gly460Trp and Negative. The percentage distribution of these genotypes among the 100 EH patients was Gly460Gly (51%), Gly460Trp (13%), Negative (36%). In a cross sectional study conducted among 432 South Indian population to assess prevalence of ADD-1 polymorphism by Ramu et al., the genotypic distribution was Gly460Gly- 59.1%, Gly460Trp-35.6%, Trp460Trp-5.3%.[74] In our study, we did not encounter Trp460Trp variant and the frequency of Gly460Trp mutant was also comparatively low.

On analysing Ethnicity based prevalence of Gly460Trp among Asians, Caucasians and Blacks, the frequency distribution noted was 55.7%, 20.9% and 6.5% respectively.[75] Hence it could be accepted that Ethnic inheritance of Gly460Trp is varied and could aid in the etio-pathogenesis of Essential Hypertension.

Among our study population, the Gly460Trp genotype was distributed predominantly in the test arm subjects (BP>140/90mmHg). The percentage prevalence of this SNP according to JNC8 classification of Hypertension was 61.5% in Stage I, 23.1% in Stage II and 15.4% in Pre-hypertension grade. Hence it could be possible that the higher grading of Hypertension among these patients was probably due to increased renal tubular reabsorption of sodium mediated by Gly460Trp.

Though the staging of Hypertension noted among Gly460Trp genotype carriers in our study group was higher, the mean duration of hypertension noted among the three genotypes among test participants was not grossly different. Gly460Trp carriers in our study did not have complications of hypertension like Coronary Artery Disease/ Renal Dysfunction or Cerebro-Vascular Accidents.

In a prospective observational study conducted by Gerhard et.al on the participants of INVEST Trial, Hypertensive Black patients with Gly460Trp mutation showed a 2.6-fold additional risk of hypertension

related cardiovascular complications, but White and Hispanic ethnic counterparts did not show such excess risk.[76] A case-control study conducted among 560 men who survived Myocardial Infarction and 646 men who underwent orthopaedic surgery in Netherland by Psaty BM et al., ADD-1 460Trp was not found to be linked to cardiovascular event risk in Hypertensive patients.[77]Our study also projects that Gly460Trp has no additional cardiovascular morbidity associated to it.

All the study participants recruited in the study were Hydrochlorthiazide naïve. On analysing their baseline antihypertensive regimen, it was interesting to note that, compared to the other two genotype counterparts (Gly460Gly & Negative), Gly460Trp carriers required more than one drug for management of their BPs irrespective of whichever arm they were allocated. Enalapril based combination with Amlodipine was used in 12 out of 13 Gly460Trp mutants.

92.3% of Gly460Trp carriers fell in the Test arm and received the intervention. This genotype based association of Gly460Trp into Interventional arm was statistically significant in our study.

Irrespective of the genotype, HCTZ add on therapy produced significant reduction in BP values in the test participants compared to the control group. In other words, HCTZ produced an additional 8mmHg decrease in SBP, 5mmHg decline in DBP and 6mmHg decrement in

MAP of test subjects compared to control subjects in our study. This decrement is in concurrence with the results of Meta-Analysis conducted by Messereli et al. with data from 14 RCTs comparing HCTZ 12.5mg dose based combination therapy efficacies.[78]

Genotype based Blood pressure changes with HCTZ usage among our study participants was found to have statistically significant inter-genotypic variation especially with regards to decrease in SBP and MAP values. In a ADD-1 genotype linkage study to establish HCTZ responsiveness in salt sensitive EH by Cusi & Bianchi et al., significant genotype based difference in BP titrations were seen.[79] This finding is consistent to our study results as well.

In the same study mentioned above by Cusi & Bianchi et al., they noted significant decline in MAP values among Gly460Trp carriers (14.7mmHg) following 2 months treatment with HCTZ than the homozygous Gly460Gly (6.8mmHg) counterparts.[79] Though in our study, Gly460Gly carriers responded in a comparable manner (delta MAP = 7.85mmHg) to their research participants, the superior response of Gly460Trp stated by their study could not be found among our study subjects.

In fact, when we tried to assess the difference in mean BP reductions between Gly460Gly and Gly460Trp Test arm participants

alone, the results were not statistically significant. Hence as per results of our study, Gly460Trp carriers did not have an added efficacy of HCTZ 12.5mg/day over Gly460Gly carriers was used as an add-on anti-hypertensive for 3 months.

Another notable finding in our study was the low frequency of distribution of Gly460Trp SNPs among our study population. Out of the 13 carriers of this mutation, 12 participants fell in the Test arm and one patient was identified among the Control arm. Hence we were unable to apply comparisons and inferential statistical tools to study impact of this genotype between test and control arms.

According to a community based sampling of 585 adults (Afro-American and Non-Hispanic White ethnic origin) on 4 weeks of HCTZ therapy, a nested case-control study based on the DBP tertiles was done by van-der Zee et al., they tried to uncover the influential pharmacogenetics guiding HCTZ responsiveness.[80]In their study, the frequency of Gly460Trp was found to be 0.13% and this SNP was allotted as rare allele frequency determinant of HCTZ responsiveness. Based on this observation, the Gly460Trp could have been a rare genetic mutation of ADD-1 among our study population as well.

Despite promising evidence supporting ADD-1 Gly460Trp mutation in Essential Hypertension and its regulatory impact on Thiazide

responsiveness from western literature, our study conducted among 100 patients of South Indian origins had low prevalence of this genetic variant hence making substantial correlation between ADD-1 Gly460Trp versus Hydrochlorthiazide efficacy difficult to establish.

CONCLUSION

Based on our study findings we conclude that,

- Three genotype classes were identified in our population: Gly460Gly (Homozygous), Gly460Trp (Heterozygous) and Negative (neither Homozygous nor Heterozygous).
- The prevalence of ADD-1 Gly460Trp mutation in our subset of population is low.
- Though the prevalence of Gly460Trp carriers were low it was significantly associated with higher staging of Essential Hypertension in spite of more than one class of pharmaco-therapeutic intervention as per our study results.
- Though higher staging classification was noted among Gly460Trp carriers of EH, propensity towards progression to CAD/CVA/CKD was not seen in our population.
- Irrespective of genotypic variations in ADD-1, Hydrochlorthiazide 12.5mg once/day addition to the other class of antihypertensive agents produced additive antihypertensive efficacy of the regimes used.

- The low frequency of Gly460Trp distribution among Control Arm in our study did not give room for application of Inferential statistics.

- Gly460Trp was a low frequency genotype trait and did not show statistically different endpoint result variations in comparison to the other 2 genotypes (Gly460Gly & Negative) in our study

LIMITATIONS OF THE STUDY

Our study results are based on a sample size of 100 Essential Hypertension patients, out of which only 50 participants with consistent 3 monthly BP values >140/90mmHg were given HCTZ 12.5mg as intervention for 3 months.

We designed the protocol with this BP cut-off as the stratification set point of allocation to have an unbiased approach to reveal the pharmacogenetic influence of ADD-1 on HCTZ action. The low frequency of Gly460Trp genotype and lack of significant association of this genotype to HCTZ efficacy could be attributed to low sample size.

ARMS-PCR (using customized primers) with gel electrophoresis and UV filter visualization of DNA bands though a sensitive method in detecting ADD-1 genotype variants is a qualitative method only. Further quantification of the genotype was not carried out in our study.

Other pharmacogentic influencers of HCTZ action were also not explored in our study. Hence the negative genotype carriers of ADD-1 polymorphism could have had other possible genetic predictors of HCTZ action.

Data regarding influence of dietary habits, alcohol intake, smoking history were not included in our study.

SCOPE FOR FUTURE STUDY

- ✓ Large sample size studies to establish association of ADD-1 polymorphism on Essential Hypertension and Antihypertensive agent responsiveness in our Ethnic group of population and establishment of Indian guidelines for management of EH
- ✓ Identification of genetic predictors of drug responsiveness could pave way in designing a personalized approach in EH management and translation into clinical practice
- ✓ If a genetic marker with significant linkage and profound impact on any of the existent antihypertensive drug class responsiveness gets uncovered in our population, it could decrease the demand on the Antihypertensive drug pipeline and also be a pharmaco-economic option from the health care system and patient perspective.

BIBILIOGRAPHY

1. Chow CK, Teo KK, Rangarajan S, Islam S, Gupta R, Avezum A, et al. PURE (Prospective Urban Rural Epidemiology) Study investigators. Prevalence, awareness, treatment and control of hypertension in rural and urban communities in high-, middle-, and low-income countries. JAMA. 2013;310:959–68. [PubMed]

2. Institute for Health Metrics and Evaluation. India high blood pressure. [Accessed on June 14, 2015]. Available from: http://www.healthmetricsandevaluation.org/search-gbd-data .

3. Johnson RJ, Feig DI, Nakagawa T, Sanchez-Lozada LG, Rodriguez-Iturbe B. Pathogenesis of essential hypertension: historical paradigms and modern insights. Journal of hypertension. 2008;26(3):381-391. doi:10.1097/HJH.0b013e3282f29876.

4. Wiener C. Harrison's principles of internal medicine. New York: McGraw-Hill, Medical Pub. Division; 2008.p. 1549-53

5. Golan D, Tashjian A. Principles of pharmacology. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2012.

6. James PA, Oparil S, Carter BL, Cushman WC, Dennison-Himmelfarb C, Handler J, et al. 2014 evidence-based guideline for the management of high blood pressure in adults: report from the panel members appointed to the Eighth Joint National Committee (JNC 8). JAMA. 2014;311: 507–520. doi: 10.1001/jama.2013.284427 [PubMed]

7. Zing W, Ferguson RK, Vlasses PH. Calcium antagonists in elderly and black hypertensive patients: therapeutic controversies. Arch Intern Med. 1991; 151: 2154–2162.

8. Taddei S, Bruno R, Ghiadoni L. The Correct Administration of Antihypertensive Drugs According to the Principles of Clinical Pharmacology. American Journal Cardiovascular Drugs. 2011;11(1):13-20.

9. Koopmans R, Insel P, Michel M. Pharmacogenetics of hypertension treatment. Pharmacogenetics. 2003;13(12):705-713.

10. Cadman P, O' Connor D. Pharmacogenomics of hypertension. Current Opinion in Nephrology and Hypertension. 2003;12(1):61-70.

11. Lin B., Nasir J., McDonald H., Graham R., Rommens J. M., Goldberg Y. P. et al. (1995) Genomic organization of the human alpha-adducin gene and its alternately spliced isoforms. Genomics 25: 93–99

12. Staessen J, Bianchi G. Adducin and hypertension. Pharmacogenomics. 2005;6(7):665-669.

13. Cusi D, Barlassina C, Azzani T, Casari G, Citterio L, Devoto M et al. Polymorphisms of α-adducin and salt sensitivity in patients with essential hypertension. The Lancet. 1997;349(9062):1353-1357.

14. Beyer K. The mechanism of action of chlorothiazide. Ann ny acad sci. 1958;71(4 chlorothiazid):363-379.

15. Moser M. Fifty Years of Thiazide Diuretic Therapy for Hypertension. Arch Intern Med. 2009;169(20):1851.

16. Freis ED, Wilson IM, Parrish AE. 19th annual meeting of the American Heart Association, Chicago, Illinois, Ott 28, 1957.

17. Smith R. Hypertension: Pathophysiology, diagnosis, and management. J. H. Laragh, B. M. Brenner, eds., Raven Press, Ltd.,

New York, 1994, 3,311 pages, $345. Drug Dev Res. 1995;35(3):183-184.

18. Veterans Cooperative Study Group on Antihypertensive Agents, Effects of treatment on morbidity in hypertension: results in patients with diastolic blood pressures averaging 115 through 129 mm Hg. JAMA 1967;202 (11) 1028- 1034

19. Hebert PRMoser MMayer JGlynn RJHennekens CH Recent evidence on drug therapy of mild to moderate hypertension and decreased risk of coronary heart disease. Arch Intern Med 1993;153 (5) 578- 581

20. ALLHAT Officers and Coordinators for the ALLHAT Collaborative Research Group, Major outcomes in high-risk hypertensive patients randomized to angiotensin-converting enzyme inhibitor or calcium channel blocker vs diuretic: the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT). JAMA 2002;288 (23) 2981- 2997

21. Chobanian AVBakris GLBlack HR et al. The Seventh Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure: the JNC 7 report. JAMA 2003;289 (19) 2560- 2572

22. Beevers DG, Lip GYH, O'Brien E. ABC of hypertension, 4th ed. London: BMJPublishing Group, 2001:12.

23. Khan J. Management of hypertension in ethnic minorities. Heart. 2005;91(8):1105-1109.

24. Comparison of propranolol and hydrochlorothiazide for thr initial treatment of hypertension. I. Results of short-term titration with

emphasis on racial differences in response. Veterans Administration Cooperative Study Group on Antihypertensive agents. JAMA: The Journal of the American Medical Association. 1982;248(16):1996-2003.

25. Nair T. European Society of Cardiology Congress 2013. Indian Heart Journal. 2013;65(6):710-711.

26. Carter BL, Ernst ME, Cohen JD : Hydrochlorothiazide versus chlorthalidone: evidence supporting their interchangeability. Hypertension 2004;43:4-9.

27. National Center for Biotechnology Information. PubChem Compound Database; CID=3639, https://pubchem.ncbi.nlm.nih.gov/compound/3639 (accessed Aug. 8, 2015).

28. Chen X, Ji ZL, Chen YZ: TTD: Therapeutic Target Database. Nucleic Acids Res. 2002 Jan 1;30(1):412-5. Pubmed

29. Temperini C, Cecchi A, Scozzafava A, Supuran CT: Carbonic anhydrase inhibitors. Comparison of chlorthalidone, indapamide, trichloromethiazide, and furosemide X-ray crystal structures in adducts with isozyme II, when several water molecules make the difference. Bioorg Med Chem. 2009 Feb 1;17(3):1214-21. Epub 2008 Dec 24. Pubmed

30. Tricarico D, Barbieri M, Mele A, Carbonara G, Camerino DC: Carbonic anhydrase inhibitors are specific openers of skeletal muscle BK channel of K+-deficient rats. FASEB J. 2004 Apr;18(6):760-1. Epub 2004 Feb 6. Pubmed

31. Goodman L, Gilman A, Brunton L, Lazo J, Parker K. Goodman & Gilman's the pharmacological basis of therapeutics. New York: McGraw-Hill; 2006.

32. Adrogue HJ, Madias NE. Sodium and potassium in the pathogenesis of hypertension. N Engl J Med. 2007;356(19):1966–1978. [PubMed]

33. Conway J, Lauwers P. Hemodynamic and hypotensive effects of long-term therapy with chlorothiazide. Circulation. 1960;21:21–27. [PubMed]

34. Lake CR, Ziegler MG, Coleman MD, Kopin IJ. Hydrochlorothiazide-induced sympathetic hyperactivity in hypertensive patients. Clin Pharmacol Ther. 1979;26(4):428–432. [PubMed]

35. Tarazi RC, Dustan HP, Frohlich ED. Long-term thiazide therapy in essential hypertension. Evidence for persistent alteration in plasma volume and renin activity. Circulation. 1970;41(4):709–717. [PubMed]

36. Holland OB, Gomez-Sanchez CE, Kuhnert LV, Poindexter C, Pak CY. Antihypertensive comparison of furosemide with hydrochlorothiazide for black patients. Arch Intern Med. 1979;139(9):1015–1021. [PubMed]

37. Shah S, Khatri I, Freis E. Mechanism of antihypertensive effect of thiazide diuretics. American Heart Journal. 1978;95(5):611-618.

38. Katsuya T, Morishita R, Rakugi H, Ogihrara T. Genetic basis of hypertension for the development of tailored medicine. Hypertension Research. 2009;32(8):643-648.

39. Kokubo Y, Kamide K, Inamoto N, Tanaka C, Banno M, Takiuchi S, Kawano Y, Tomoike H, Miyata T. Identification of 108 SNPs in TSC, WNK1, and WNK4 and their association with hypertension in a Japanese general population. J Hum Genet 2004;49: 507–515.

40. Okamoto K, Aoki K. Development of a strain of spontaneously hypertensive rats. Jap Circ J 1963;27:282-93.

41. Badyal DK, Lata H, Dadhich AP. Animal models of hypertension and effect of drugs. Indian Journal of Pharmacology. 2003;35: 349-62.

42. Bianchi, G., Tripodi, G., Casari, G., Salardi, S., Barber, B. R., Garcia, R., Leoni, P., Torielli, L., Cusi, D., Ferrandi, M., Pinna, L. A., Baralle, F. E., Ferrari, P. Two point mutations within the adducin genes are involved in blood pressure variation. Proc. Nat. Acad. Sci. 91: 3999-4003, 1994.

43. Bianchi G, Tripodi MG, Casari G, Torielli L, Cusi D, et al. (1995) alphaadducin may control blood pressure both in rats and humans. Clin Exp Pharmacol Physiol Suppl 22: S7–9.

44. Efendiev, R., Krmar, R. T., Ogimoto, G., Zwiller, J., Tripodi, G., Katz, A. I., Bianchi, G., Pedemonte, C. H., Bertorello, A. M. Hypertension-linked mutation in the adducin alpha-subunit leads to higher AP2-mu-2 phosphorylation and impaired Na+, K+-ATPase trafficking in response to GPCR signals and intracellular sodium. Circ. Res. 95: 1100-1108, 2004.

45. Bennett V, Stenbuck P. The membrane attachment protein for spectrin is associated with band 3 in human erythrocyte membranes. Nature. 1979;280(5722):468-473.

46. Matsuoka Y, Li X, Bennett V. Adducin: structure, function and regulation. CMLS, Cell Mol Life Sci. 2000;57(6):884-895.

47. Casari G, Barlassina C, Cusi D, Zagato L, Muirhead R, et al. (1995) Association of the alpha-adducin locus with essential hypertension. Hypertension 25:320–326.

48. Cusi D, Barlassina C, Azzani T, Casari G, Citterio L, et al. (1997) Polymorphisms of alpha-adducin and salt sensitivity in patients with essential hypertension. Lancet 349: 1353–1357.

49. Manunta P, Barlassina C, Bianchi G (1998) Adducin in essential hypertension.FEBS Lett 430: 41–44

50. Manunta P, Cusi D, Barlassina C, Righetti M, Lanzani C, et al. (1998) Alpha adducin polymorphisms and renal sodium handling in essential hypertensive patients.Kidney Int 53: 1471–1478.

51. Allayee H, de Bruin T, Michelle Dominguez K, Cheng L, Ipp E, Cantor R et al. Genome Scan for Blood Pressure in Dutch Dyslipidemic Families Reveals Linkage to a Locus on Chromosome 4p. Hypertension. 2001;38(4):773-778.

52. Sciarrone MT, Stella P, Barlassina C, Manunta P, Lanzani C, et al. (2003) ACE and alpha-adducin polymorphism as markers of individual response to diuretic therapy. Hypertension 41: 398–403.

53. Cusi D, Barlassina C, Azzani T, Casari G, Citterio L, Devoto M, et al.Polymorphisms of alpha-adducin and salt sensitivity in patients with essential hypertension. Lancet 1997; 349:1353–1357.

54. Glorioso N, Manunta P, Filigheddu F, Troffa C, Stella P, Barlassina C, et al.The role of alpha-adducin polymorphism in blood pressure and sodium handling regulation may not be

excluded by a negative association study. Hypertension 1999; 34:649–654.

55. Liu K, Liu J, Huang Y, Liu Y, Lou Y, Wang Z et al. Alpha-Adducin Gly460Trp Polymorphism and Hypertension Risk: A Meta-Analysis of 22 Studies Including 14303 Cases and 15961 Controls. PLoS ONE. 2010;5(9):e13057.

56. Alam S, Liyou N, Davis D, Tresillian M, Johnson A. The 460Trp polymorphism of the human α-adducin gene is not associated with isolated systolic hypertension in elderly Australian Caucasians. J Hum Hypertens. 2000;14(3):199-203.

57. Ciechanowicz A, Widecka K, Drozd R, Adler G, Cyryłowski L, Czekalski S. Lack of Association between Gly460Trp Polymorphism of Alpha-Adducin Gene and Salt Sensitivity of Blood Pressure in Polish Hypertensives. Kidney Blood Press Res. 2001;24(3):201-206.

58. Li Y. α-Adducin Gly460Trp Gene Mutation and Essential Hypertension in a Chinese Population: A Meta-Analysis including 10960 Subjects. PLoS ONE. 2012;7(1):e30214.

59. Narita I, Goto S, Saito N, Song J, Ajiro J, Sato F et al. Interaction Between ACE and ADD1 Gene Polymorphisms in the Progression of IgA Nephropathy in Japanese Patients. Hypertension. 2003;42(3):304-309.

60. Beeks E, van der Klauw M, Kroon A, Spiering W, Fuss-Lejeune M, de Leeuw P. -Adducin Gly460Trp Polymorphism and Renal Hemodynamics in Essential Hypertension. Hypertension. 2004;44(4):419-423.

61. Ramu P, Umamaheswaran G, Shewade DG, Swaminathan RP, Balachander J, Adithan C. Gly460Trp polymorphism of the *ADD1* gene and essential hypertension in an Indian population: A meta-analysis on hypertension risk. *Indian Journal of Human Genetics*. 2010;16(1):8-15. Doi:10.4103/0971-6866.64938.

62. P. Ferrari, L. Torielli, M. Ferrandi, et al., PST 2238: a new antihypertensive compound that antagonizes the long-term pressor effect of ouabain, J. Pharm. Exp.Ther. 285 (1) (1998) 83–94.

63. P. Ferrari, M. Ferrandi, G. Tripodi, et al., PST 2238: a new antihypertensive compound that modulates Na+–K+ATPase in genetic hypertension, J. Pharm. Exp.Ther. 288 (3) (1999) 1074–1083.

64. M. Ferrandi, I. Molinari, P. Barassi, E. Minotti, G. Bianchi, P. Ferrari, Organ hypertrophic signaling within caveolae membrane subdomains triggered by ouabain and antagonized by PST2238, J. Biol. Chem. 279 (32) (2004) 33306–33314.

65. Bianchi G, Ferrari P, Staessen J. Adducin Polymorphism: Detection and Impact on Hypertension and Related Disorders. Hypertension. 2005;45(3):331-340.

66. P. Ferrari, M. Ferrandi, L. Torielli, M.G. Tripodi, P. Melloni, G. Bianchi, PST 2238: a new antihypertensive compound that modulates Na+–K+ATPase and antagonizes the pressor effect of OLF, Cardiovasc. Drug Rev. 17 (1) (1999) 39–57.

67. M. Ferrandi, P. Barassi, E. Minotti, et al., PST 2238: a new antihypertensive compound that modulates renal Na–K pump function without diuretic activity in Milan hypertensive rats, J. Cardiovasc. Pharmacol. 40 (6) (2002) 881–889.

68. J.A. Staessen, T. Kuznetsova, R. Acceto, et al., OASIS-HT: design of a pharmacogenomic dose-finding study, Pharmacogenomics 6 (2005) 755–775.

69. Meckley L, Veenstra D. Screening for the alpha-adducin Gly460Trp variant in hypertensive patients: a cost-effectiveness analysis. Pharmacogenetics and Genomics. 2006;16(2):139-147.

70. Lynch AI, Boerwinkle E, Davis BR, et al. Pharmacogenetic association of the NPPA T2238C genetic variant with cardiovascular disease outcomes in patients with hypertension. JAMA 2008; 299:296–307.

71. Khoury MJ, Berg A, Coates R, et al. The evidence dilemma in genomic medicine. Health Aff (Millwood) 2008; 27:1600–1611.

72. Arnett D, Claas S, Lynch A. Has pharmacogenetics brought us closer to 'personalized medicine' for initial drug treatment of hypertension?. Current Opinion in Cardiology. 2009;24(4):333-339.

73. Staessen J, Thijs L, Stolarz-Skrzypek K, Bacchieri A, Barton J, Espositi E et al. Main results of the Ouabain and Adducin for Specific Intervention on Sodium in Hypertension Trial (OASIS-HT): a randomized placebo-controlled phase-2 dose-finding study of rostafuroxin. Trials. 2011;12(1):13.

74. Ramu P, Umamaheswaran G, Shewade DG, Swaminathan RP, Balachander J, Adithan C. Gly460Trp polymorphism of the *ADD1* gene and essential hypertension in an Indian population: A meta-analysis on hypertension risk. *Indian Journal of Human Genetics*. 2010;16(1):8-15. Doi:10.4103/0971-6866.64938.

75. Liu K, Liu J, Huang Y, Liu Y, Lou Y, Wang Z et al. Alpha-Adducin Gly460Trp Polymorphism and Hypertension Risk: A Meta-Analysis of 22 Studies Including 14303 Cases and 15961 Controls. Plos ONE. 2010;5(9):e13057.

76. α-adducin polymorphism associated with increased risk of adverse cardiovascular outcomes: results from invest-genes *am heart j*. 2008 august ; 156(2): 397–404. Oi:10.1016/j.ahj.2008.03.007.

77. Psaty B, Doggen C, Vos H, Vandenbroucke J, Rosendaal F. Association of the α-adducin polymorphism with blood pressure and risk of myocardial infarction. J Hum Hypertens. 2000;14(2):95-97.

78. Messerli F, Makani H, Benjo A, Romero J, Alviar C, Bangalore S. Antihypertensive Efficacy of Hydrochlorothiazide as Evaluated by Ambulatory Blood Pressure Monitoring. Journal of the American College of Cardiology. 2011;57(5):590-600.

79. Cusi D, Barlassina C, Azzani T, Casari G, Citterio L, Devoto M et al. Polymorphisms of α-adducin and salt sensitivity in patients with essential hypertension. The Lancet. 1997;349(9062):1353-1357.

80. Maitland-van der Zee A, Turner S, Schwartz G, Chapman A, Klungel O, Boerwinkle E. A multilocus approach to the antihypertensive pharmacogenetics of hydrochlorothiazide. Pharmacogenetics and Genomics. 2005;15(5):287-293.

S.NO	Age	Sex	BMI	HTN	Baseline			ARM	ADD-1	Baseline Drugs			Test Drug	Co-Morbidities (Years)						Con-comittant Medication							Follow-up		
				Y	SBP	DBP	MAP			Enalapril	Amlodipine	Atenolol	HCTZ	DM	CLD	CKD	CAD	CVA	Hypothyroidism	Metformin	Glipizide	Glimeperide	Insulin	Atorvastatin	Aspirin	Thyroxine	SBP	DBP	MAP
					mm Hg	mm Hg	mm Hg			2.5mg	2.5mg	50mg	12.5 mg							500mg	5mg	2mg	U	10mg	75 mg	50mcg	mm Hg	mm Hg	mm Hg
1	38	F	23.4	2	136	84	101.3	C	Gly/Gly	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	140	80	100
2	49	M	24.8	1	130	80	96.7	C	Gly/Gly	0	2	0	0	0	0	0	0	0	0	3	2	0	0	1	0	0	130	84	99.3
3	55	F	26.4	9	130	90	103.3	T	Gly/Trp	2	0	0	1	10	0	0	0	0	0	0	0	0	0	0	0	0	130	80	96.7
4	62	F	23.4	10	130	70	90	C	Gly/Gly	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	130	80	96.7
5	55	F	28.6	10	160	100	120	T	Gly/Gly	0	4	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	140	90	106.7
6	41	F	26.8	1	130	90	103.3	T	-	0	1	0	1	0	0	0	0	0	0	2	1	0	0	0	0	0	130	80	96.7
7	45	F	23.2	10	150	90	110	T	Gly/Gly	2	0	0	1	4	0	0	0	0	0	2	0.5	0	0	0	0	0	140	86	104
8	37	F	24	2	150	96	114	T	Gly/Gly	1	0	0	1	2	0	0	0	0	0	0	0	1	0	0	0	1	138	90	106
9	48	F	26.4	9	160	96	117.3	T	Gly/Trp	0	2	1	1	0	0	0	0	0	10	0	0	0	0	0	0	0	140	80	100
10	44	F	23.8	4	160	100	120	T	Gly/Gly	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	144	90	108
11	56	F	27.4	3	110	70	83.3	C	-	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	120	80	93.3
12	52	F	26.2	7	130	80	96.7	C	Gly/Gly	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	130	80	96.7
13	45	F	25.8	1	130	80	96.7	C	Gly/Gly	0	2	1	0	0	0	0	0	0	8	0	0	0	0	0	0	0	130	86	100.7
14	57	F	23.4	6	160	100	120	T	Gly/Gly	2	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	140	92	108
15	58	M	22.6	8	160	100	120	T	Gly/Gly	4	0	0	1	0	0	0	0	0	0	2	0	0	0	0	0	0	140	90	106.7
16	54	F	26.4	10	150	96	114	T	-		0	1	1	6	0	0	0	0	0	0	0	0	0	0	0	0	140	90	106.7
17	36	M	23.8	5	140	100	113.3	T	Gly/Trp	2	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	130	90	103.3
18	47	M	24.8	7	130	80	96.7	C	Gly/Gly	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	130	80	96.7
19	60	M	23.4	12	166	100	122	T	Gly/Gly	2	4	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	148	90	109.3
20	60	M	25	12	150	100	116.7	T	-	2	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	138	88	104.7
21	64	F	27.2	10	130	80	96.7	C	Gly/Gly	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	130	80	96.7
22	50	F	24.4	9	140	100	113.3	T	Gly/Gly	0	4	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	128	90	102.7
23	60	F	23.6	10	130	86	100.7	C	-	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	130	80	96.7
24	58	F	25.8	9	140	90	106.7	T	-	2	2	0	1	0	0	0	0	0	0	2	0	2	0	0	0	0	140	84	102.7

25	40	F	22.6	2	130	80	96.7	C	-	2	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	130	80	96.7
26	52	F	21.8	12	130	80	96.7	C	Gly/Gly	2	2	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	130	80	96.7
27	59	F	22.5	6	140	90	106.7	T	Gly/Gly	2	2	0	1	2	0	0	0	0	0	3	2	0	0	0	0	0	130	90	103.3
28	58	F	22.8	2	180	100	126.7	T	Gly/Gly	2	4	0	1	8	0	0	0	0	0	3	0	0	30	0	0	0	160	90	113.3
29	48	F	23.2	7	130	80	96.7	C	Gly/Gly	4	4	0	0	3	0	0	0	0	0	0	0	0	0	1	0	0	130	80	96.7
30	45	F	23.4	3	110	70	83.3	C	-	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	120	80	93.3
31	49	F	22.8	3	120	80	93.3	C	Gly/Gly	2	2	0	0	0	0	0	0	0	0	1	0	2	0	0	0	0	120	80	93.3
32	52	F	24.6	6	160	80	106.7	T	Gly/Trp	4	4	0	1	2	0	0	0	0	0	2	0	2	0	0	0	0	140	80	100
33	55	F	25.8	8	150	90	110	T	Gly/Gly	4	0	0	1	15	0	0	0	0	0	2	0	0	0	0	0	0	140	90	106.7
34	45	F	23.5	1	130	70	90	C	Gly/Gly	2	4	1	0	1	0	0	0	0	0	2	0	2	0	0	0	0	130	80	96.7
35	50	F	21.4	5	150	100	116.7	T	Gly/Gly	2	2	0	1	2	0	0	0	0	0	2	0	0	0	0	0	0	140	90	106.7
36	52	F	22	7	140	100	113.3	T	-	0	2	1	1	2	0	0	0	0	0	0	0	0	0	0	0	0	140	90	106.7
37	50	F	23.2	1	110	80	90	C	Gly/Gly	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	120	80	93.3
38	50	F	26.4	8	150	100	116.7	T	-	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	140	90	106.7
39	54	F	21	2	140	100	113.3	T	Gly/Trp	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	140	90	106.7
40	53	F	22.4	5	130	80	96.7	C	-	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	130	80	96.7
41	53	F	19.8	5	130	70	90	C	-	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	130	80	96.7
42	61	F	24	5	160	90	113.3	T	-	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	146	86	106
43	58	F	23.6	10	130	80	96.7	C	-	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	130	80	96.7
44	63	M	20.4	8	130	86	100.7	C	-	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	130	90	103.3
45	58	M	21.8	8	140	80	100	T	Gly/Trp	4	4	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	140	80	100
46	55	M	23.4	6	150	90	110	T	Gly/Gly	2	4	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	140	90	106.7
47	50	M	23.5	10	140	90	106.7	T	-	2	2	0	1	0	0	0	2	0	0	3	2	0	0	0	0	0	140	80	100
48	60	M	22.8	8	150	100	116.7	T	Gly/Gly	2	4	1	1	10	0	0	0	0	0	3	1	0	0	0	1	0	140	90	106.7
49	48	M	21.9	1	150	90	110	T	Gly/Gly	4	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	140	80	100
50	43	M	19.6	8	140	80	100	T	Gly/Gly	4	2	0	1	0	0	0	7	0	0	0	0	0	0	0	0	0	140	80	100
51	60	M	20.8	12	160	100	120	T	-	2	4	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	138	90	106
52	55	M	26.4	10	150	90	110	T	Gly/Trp	2	1	0	1	10	0	0	0	0	0	0	0	0	0	0	0	0	140	80	100
53	55	M	24.3	6	136	84	101.3	C	Gly/Gly	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	130	80	96.7
54	51	M	22.8	5	130	80	96.7	C	-	0	2	1	0	2	0	0	0	0	0	2	1	0	0	0	0	0	130	80	96.7
55	52	M	26.4	12	150	90	110	T	Gly/Trp	2	2	0	1	4	0	0	0	0	0	3	0	2	0	1	0	0	140	90	106.7
56	47	M	25.5	3	130	70	90	C	-	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	130	80	96.7

57	54	M	24.8	8	130	80	96.7	C	Gly/Gly		0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	130	80	96.7
58	44	F	24.6	4	150	90	110	T	Gly/Gly	2	2	0	1	0	0	0	0	0	0	0	0	0	1	0	0	140	90	106.7
59	52	F	22.8	8	150	90	110	T	Gly/Gly	1	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	140	86	104
60	52	F	23.4	6	120	86	97.3	C	Gly/Gly	2	4	0	0	0	0	0	0	0	0	0	0	0	1	0	0	130	86	100.7
61	55	M	22.4	5	160	96	117.3	T	Gly/Gly	2	2	0	1	2	0	0	0	0	0	2	1	0	0	0	0	154	90	111.3
62	48	F	21.8	1	130	80	96.7	C	-	2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	130	80	96.7
63	50	M	19.8	4	130	70	90	C	Gly/Gly	0	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	130	80	96.7
64	51	M	18.4	9	130	80	96.7	C	-	2	0	0	0	4	0	0	0	0	0	2	0	1	0	0	0	130	80	96.7
65	50	M	23.2	4	160	100	120	T	Gly/Trp	2	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	150	90	110
66	55	F	22.4	5	130	80	96.7	C	-	2	0	0	0	2	0	0	0	0	0	2	2	0	0	0	0	130	80	96.7
67	59	F	21.8	8	130	80	96.7	C	Gly/Gly	2	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	130	80	96.7
68	52	F	24.8	2	150	96	114	T	-	2	2	0	1	0	0	0	0	0	0	0	0	0	1	0	0	150	90	110
69	55	F	26.8	5	140	100	113.3	T	Gly/Gly	2	4	0	1	0	0	0	0	0	0	0	0	0	0	0	0	140	90	106.7
70	51	M	22.4	8	130	80	96.7	C	-	4	4	0	0	8	0	0	0	0	0	3	0	1	0	0	0	130	80	96.7
71	44	F	23.4	3	130	80	96.7	C	Gly/Gly	1	0	0	0	6	0	0	0	0	0	2	2	0	0	0	0	130	80	96.7
72	58	F	23.6	6	150	100	116.7	T	-	2	2	0	1	2	0	0	0	0	0	2	0	0	0	1	0	0	150	90	110
73	55	M	22.8	5	130	80	96.7	C	-	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	130	80	96.7
74	54	M	25.8	3	140	100	113.3	T	Gly/Gly	4	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	136	96	109.3
75	56	M	26.3	8	130	86	100.7	C	-	2	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	130	86	100.7
76	51	M	26.4	6	140	90	106.7	T	Gly/Trp	2	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	140	90	106.7
77	56	M	23.4	7	150	100	116.7	T	Gly/Gly	0	2	1	1	4	0	0	0	0	0	3	0	0	0	1	0	140	90	106.7
78	55	M	24.8	5	130	80	96.7	C	-	2	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	130	80	96.7
79	42	M	25.4	2	130	80	96.7	C	Gly/Gly	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	130	80	96.7
80	45	M	26.4	2	180	100	126.7	T	Gly/Gly	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	160	90	113.3
81	40	M	23.8	2	130	80	96.7	C	-	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	130	80	96.7
82	51	M	22.6	3	110	70	83.3	C	Gly/Gly	0	2	0	0	8	0	0	0	0	0	3	2	0	0	0	0	120	80	93.3
83	60	M	21.2	7	160	80	106.7	T	Gly/Gly	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	146	80	102
84	54	M	23.8	6	160	100	120	T	Gly/Gly	0	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0	140	90	106.7
85	48	M	23.5	8	130	80	96.7	C	Gly/Gly	4	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	130	80	96.7
86	59	M	24.6	11	130	70	90	C	-	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	130	80	96.7
87	56	M	25.4	8	150	100	116.7	T	Gly/Trp	2	4	0	1	0	0	0	0	0	0	0	0	0	0	0	0	150	90	110
88	44	M	22.5	4	130	70	90	C	-	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	140	80	100

89	56	F	24.6	10	110	80	90	C	Gly/Gly	2	4	1	0	0	0	0	0	0	0	0	0	0	1	0	0	120	80	93.3
90	52	M	21	6	120	80	93.3	C	-	4	0	0	0	4	0	0	0	0	0	2	0	0	0	1	0	0	120	80	93.3
91	51	M	23.6	4	130	80	96.7	C	Gly/Gly	4	2	0	0	4	0	0	0	0	0	2	0	2	0	0	0	0	130	80	96.7
92	43	M	18.6	3	130	80	96.7	C	Gly/Gly	2	4	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	130	80	96.7
93	52	F	22.4	8	140	90	106.7	T	Gly/Gly	2	1	0	1	2	0	0	0	0	0	2	0	0	0	0	0	0	140	90	106.7
94	56	M	23.8	3	160	90	113.3	T	Gly/Gly	4	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	154	88	110
95	48	M	24.6	2	130	80	96.7	C	-	4	4	0	0	2	0	0	0	0	0	2	0	0.5	0	0	0	0	130	80	96.7
96	54	M	23.8	5	130	86	100.7	C	-	2	4	0	0	6	0	0	0	0	0	3	2	0	0	1	0	0	130	80	96.7
97	60	F	24.6	12	130	80	96.7	C	-	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	130	80	96.7
98	57	M	26	7	150	90	110	T	Gly/Trp	2	4	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	146	90	108.7
99	59	M	25.8	8	130	80	96.7	C	Gly/Trp	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	130	80	96.7
100	60	M	22.6	10	120	80	93.3	C	-	4	2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	120	80	93.3

α-ADDUCIN POLYMORPHISM GENOTYPING – MATERIALS AND METHODOLOGY

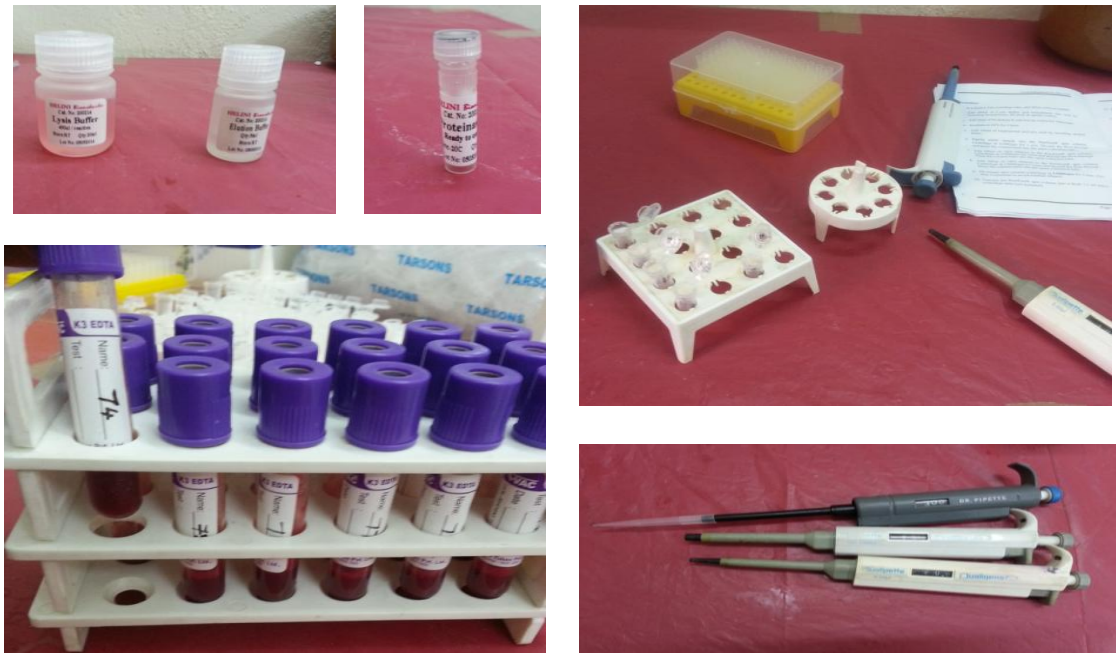


Fig.1: (Clockwise from top left) Articles for DNA extraction – Lysis Buffer, Elution Buffer, Proteinase K; working rack for the processing with protocol pamphlet by the side, Micro pipettes, Blood samples collected in EDTA vaccutainers.

Fig.2: Instruments needed for DNA extraction

1. **Water Bath**
2. **Vortex Shaker**
3. **Refrigerated centrifuge**

Fig.3: (Clockwise from top left) : Laminar flow chamber for sterile work area, PCR Machine, Gel Electrophoresis visualization unit, PCR display screen with α-Adducin program settings.

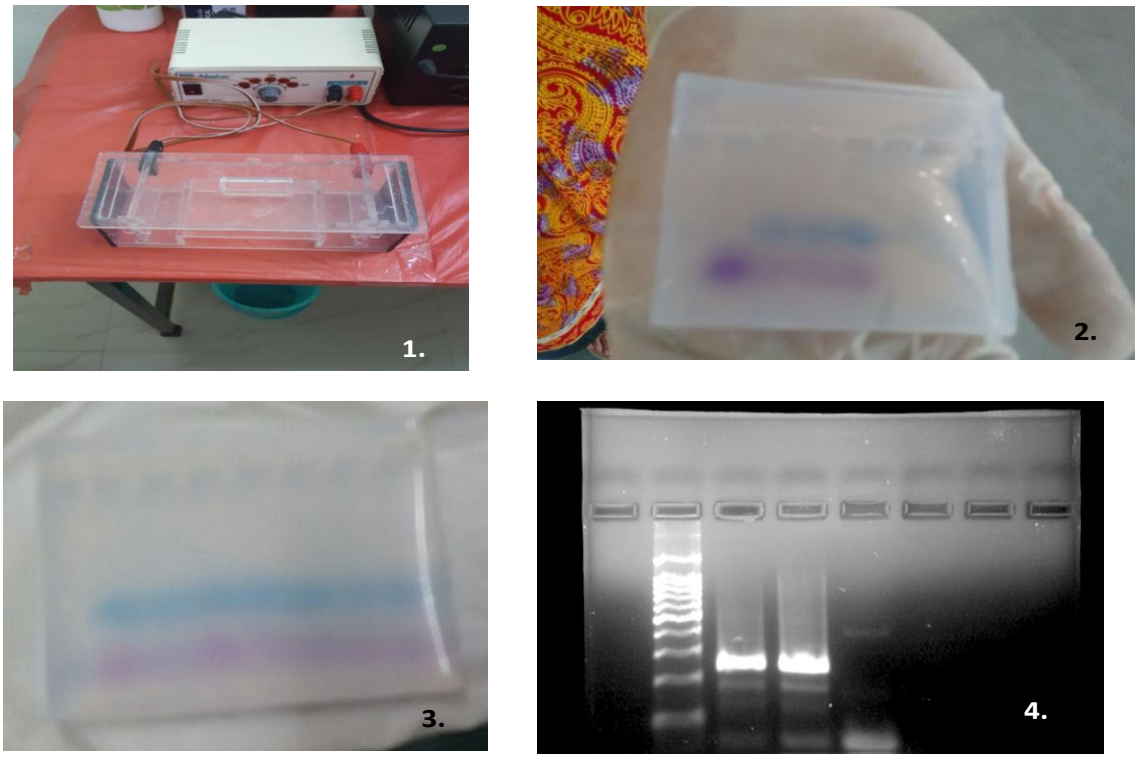


Fig.4: (1) Submarine type gel electrophoresis apparatus,

(2) Cast gel after completion of electrophoresis,

(3) Cast and Run Gel showing pink (Ethidium bromide) & blue (gel loading dye) indicator lines,

(4) Visualization of DNA bands with UV light. (Standardization done at 60ºC)

PROFORMA

REF. NO:

NAME: AGE/SEX:

CONTACT :

HYPERTENSION HISTORY:

DURATION:

CURRENT ANTIHYPERTENSIVE MEDICATION :

BP RECORDS OF PAST 3 MONTHS:

PAST MEDICAL HISTORY:

DM:

LIVER DISEASE:

RENAL DISEASE:

ALLERGIES:

PAST H/O SIGNIFICANT HOSPITALIZATION/ SURGERY:

BASELINE INVESTIGATION:

CBC	LFT	RFT	Glycemic/Lipid Profile
HB:	S.Bil:	Urea:	RBS:
TC:	SGOT:	Creat:	T.Cholesterol:
DC:	SGPT:	Na^+: K^+:	Triglycerides:
ESR:	SAP:		
PLT:	S.Protein:		

AVERAGE 3 MONTH BP VALUE :

ALLOCATION : Control/Test

IF TEST, T.HYDROCHLORTHIAZIDE 12.5mg OD *3months

Drug Issue Date	BP recording during visit	Reported ADR	Lab investigation
1.			5ml of venous blood in EDTA vaccutainer for genotyping
2.			-
3.			Issue Lab slip
4. End of study drug			Verify lab parameters

FOLLOW-UP LAB INVESTIGATION:

CBC	LFT	RFT	Glycemic/Lipid Profile
HB:	S.Bil:	Urea:	RBS:
TC:	SGOT:	Creat:	T.Cholesterol:
DC:	SGPT:	Na^+: K^+:	Triglycerides:
ESR:	SAP:		
PLT:	S.Protein:		

GENOTYPING RESULT:

PATIENT INFORMATION SHEET - நோயாளி தகவல் தாள்

α Adducin (அல்பா அடுசின்) என்பது என் உடம்பின் மரபணுவில் இருந்து உருவாக்கப்படும் ஒரு புரதம். இந்த புரதம் என் சிறுநீரகத்தின் வழியாக உடம்பில் உப்புத் தங்க காரணமாக உள்ளதா என்பதையும் அதன் காரணமாக எனக்கு கொடுக்கப்படும் இரத்த அழுத்தத்திற்கான மருந்தின் ஆற்றலுக்கு ஏதேனும் தாக்கம் ஏற்படுகிறதா என்பதை அறிந்து கொள்வதற்காக இந்த ஆய்வு மேற்கொள்ளப்படுகிறது.

இந்த ஆய்விற்காக நான் முழு இரத்த பரிசோதனை மற்றும் மரபணுவில் அல்பா அடுசினை கண்டுபிடிக்க 5 மில்லி இரத்தம் கொடுக்க வேண்டும். இந்த இரத்த பரிசோதனை என் உடல் ஆரோக்கியம் இரத்த அழுத்தத்தினால் பாதிக்கப்பட்டிருகிறதா என்பதை குறிக்கும். அந்த பரிசோதனை சீராக இருந்தால் மட்டுமே எனக்கு இந்த ஆய்வில் பங்குபெற அனுமதி கொடுக்கப்படும்.

எனது இரத்த அழுத்தம் 140/90 என்ற அளவிற்கு மேல் இருந்தால் எனக்கு Hydrochlorthiazide என்னும் மருந்து நான் உண்ணும் மற்ற இரத்த அழுத்த மருந்துக்களுடன் எனக்கு 3 மாத காலம் ஆய்வாளரால் கொடுக்கப்படும். இந்த மருந்தினால் என் இரத்த அழுத்தம் குறைய வாய்ப்பு உண்டு. இந்த மருந்தினால் சில பின்விளைவுகள் ஏற்ப்பட நேரிடலாம். இந்த பின்விளைவுகள் என் இரத்தத்தில் உள்ள சர்க்கரை, கொழுப்பு, உரிக் அசிட், கால்சியம் ஆகியவற்றை அதிகப்படுத்தும் தன்மை உடையது.

*இந்த நகலில் உள்ள ஏதேனும் விவரங்களின் மீது உங்களுக்கு சந்தேகம் இருப்பின், ஆய்வாளரை அணுகி உங்கள் கேள்விகளை கேட்டு அறிந்துகொள்ளுங்கள்.

*இந்த நகலில் உள்ள அணைத்து விவரங்களும் உண்மையானவை. இதைப் படித்து நன்கு உணர்த்த பின், உங்களது பரிபூரண சம்மதம் அளித்து, எந்த வித ஆய்வாளரின் தூண்டுதலின் காரணமுமின்றி இந்த ஆய்வில் பங்கெடுத்து கொள்ளுங்கள்.

Patient Consent Form

Study Title: A study to assess association of α-Adducin polymorphism in essential hypertension and its impact on responsiveness to hydrochlorthiazide in patients attending hypertension clinic in a tertiary care hospital

Study Center: Kilpauk Medical College, Chennai-10

Patient Name: O.P. No.:

Patient Age/sex:

I confirm that I have understood the purpose and procedure of the above study. I had the opportunity to ask questions and all my doubts have been answered satisfactorily.

I understand that my participation in the study is voluntary and that I am free to withdraw at any time without my legal rights being affected.

I understand that the members of the ethical committee and the investigator involved in the study will not need my permission to look at my health records, both in respect to the current study and any other further research that may be conducted in relation to it. However, I understand that my identity will not be revealed in any information released to third parties or published, unless as required under the law. I agree not to restrict the use of any data or results that may arise from this study.

I hereby consent to participate in this study.

I hereby give permission to undergo complete clinical examination and diagnostic tests including withdrawal of 5ml of blood at the beginning and end of the study.

Patient Signature/ Thumb Impression:

Patient Name and address:

Witness Signature/ Thumb Impression:

Witness Name and address:

Investigator's Signature:

Name of the Investigator:

Place:

Date:

நோயாளி ஒப்புதல் படிவம்

உயர் இரத்த அழுத்தம் நோயாளிகளுக்கு α- Adducin பாலிமார்பிஸம் மற்றும் அதன் காரணமாக Hydrochlorthiazide மருந்து செயல்திறனுக்கு உள்ள மாற்றம் குறித்த ஆய்வு

ஆராய்ச்சி மையம் - அரசு கீழ்பாக்கம் மருத்துவக் கல்லூரி மருத்துவமனை

நோயாளியின் பெயர் : நோயாளியின் வயது : ஆ/பெ

பதிவு எண் : முகவரி :

1. மேற்குறிப்பிட்டுள்ள ஆராய்ச்சியின் நோக்கத்தையும் பயனையும் முழுவதுமாக புரிந்து கொண்டேன். மேலும் எனது அனைத்து சந்தேகங்களையும் கேட்டு அதற்கான விளக்கங்களையும் தெளிவுபடுத்திக்கொண்டேன்.
2. மேலும் இந்த ஆராய்ச்சிக்கு எனது சொந்த விருப்பத்தின் பேரில் பங்கேற்கிறேன் என்றும், மேலும் எந்த நேரத்திலும் எவ்வித முன்னறிவிப்புமின்றி இந்த ஆராய்ச்சியிலிருந்து விலக முழுமையான உரிமை உள்ளதையும், இதற்கு எவ்வித சட்ட பிணைப்பும் இல்லை என்பதையும் அறிவேன்.
3. ஆராய்ச்சியாளரே, ஆராய்ச்சி உதவியாளரோ, ஆராய்ச்சி உபயத்தாரோ, ஆராய்ச்சி பேராசிரியரோ, ஒழுங்குநெறி செயற்குழு உறுப்பினர்களோ எப்போது வேண்டுமானாலும் எனது அனுமதியின்றி எனது உள்நோயாளி பதிவுகளை இந்த ஆராய்ச்சிக்காகவோ அல்லது எதிர்கால பிற ஆராய்ச்சிகளுக்காகவோ பயன்படுத்திக் கொள்ளலாம் என்றும் மேலும் இந்த நிபந்தனை நான் இவ்வாராய்ச்சியிலிருந்து விலகினாலும் தரும் என்றும் ஒப்புக் கொள்கிறேன். ஆயினும் எனது அடையாளம் சம்பந்தப்பட்ட எந்த பதிவுகளும் (சட்டபூர்வமான தேவைகள் தவிர) வெளியிடப்படமாட்டாது என்ற உறுதிமொழியின் பெயரில் இந்த ஆராய்ச்சியிலிருந்து கிடைக்கப்பெறும் முடிவுகளைவெளியிட மறுப்பு தெரிவிக்கமாட்டேன் என்று உறுதியளிக்கின்றேன்.
4. இந்த ஆராய்ச்சிக்கு நான் முழு மனதுடன் சம்மதிக்கின்றேன் என்றும், மேலும் ஆராய்சிசிக் குழுவினர் எனக்க அளிக்கும் அறிவுரைகளை தவறாது பின்பற்றுவேன் என்றும் உறுதியளிக்கின்றேன்.
5. இந்த ஆராய்சிசிக்குத் தேவைப்படும் அனைத்து மருத்துவப் பரிசோதனைகளுக்கும் ஒத்துழைப்பு தருவேன் என்று உறுதியளிக்கின்றேன்.
6. இந்த ஆராய்ச்சியில் சக்கரை நோய்கான பரிசோதனைகளுக்கும், மேலும் மரபணு சோதனையும் மேற்கொள்ளப்படுகிறது என்பதை ஆராய்ச்சியாளர் மூலம் அறிந்து கொண்டேன். மரபணு சோதனைக்கும் எனது முழு ஒப்புதலை தருகிறேன்.
7. இந்த ஆராய்சிக்கு யாருடைய வற்புறுத்தலுமின்றி எனது சொந்த விருப்பத்தின் பேரிலும் சுய அறிவுடனும் முழுமனதுடனும் சம்மதிக்கின்றேன் என்று இதன் மூலம் ஒப்புக் கொள்கிறேன்.

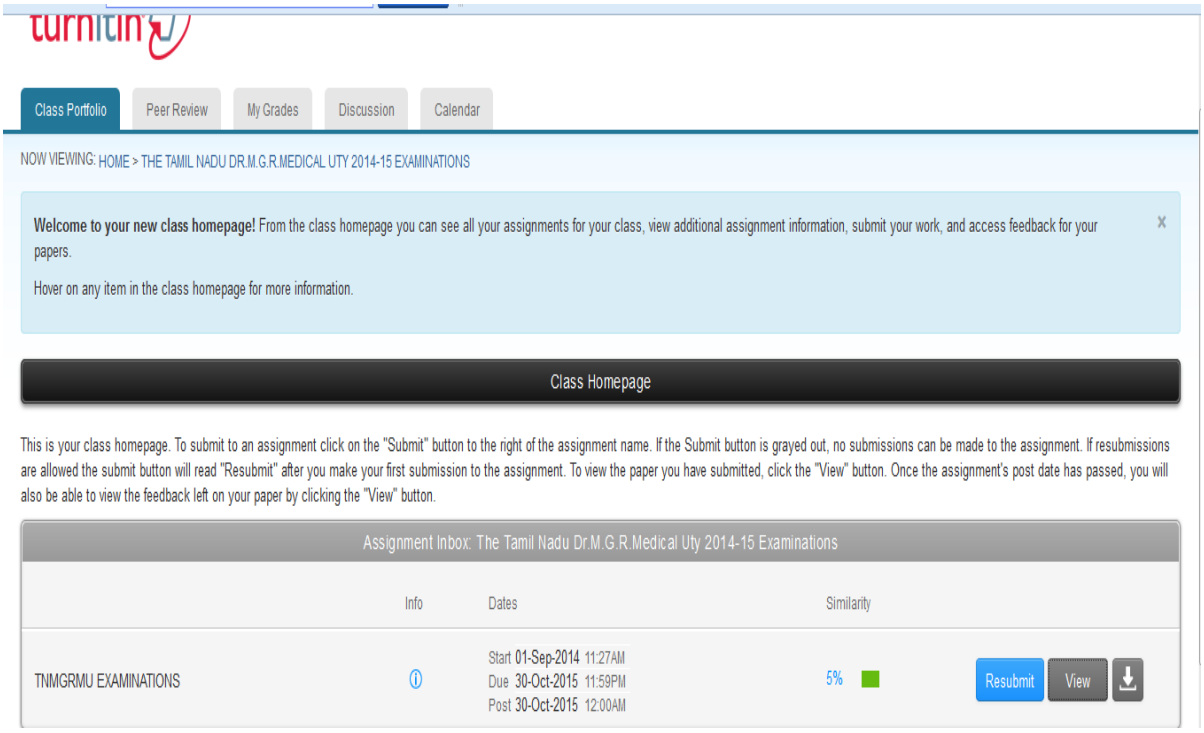
ஆராய்ச்சியாளரின் கையொப்பம் :

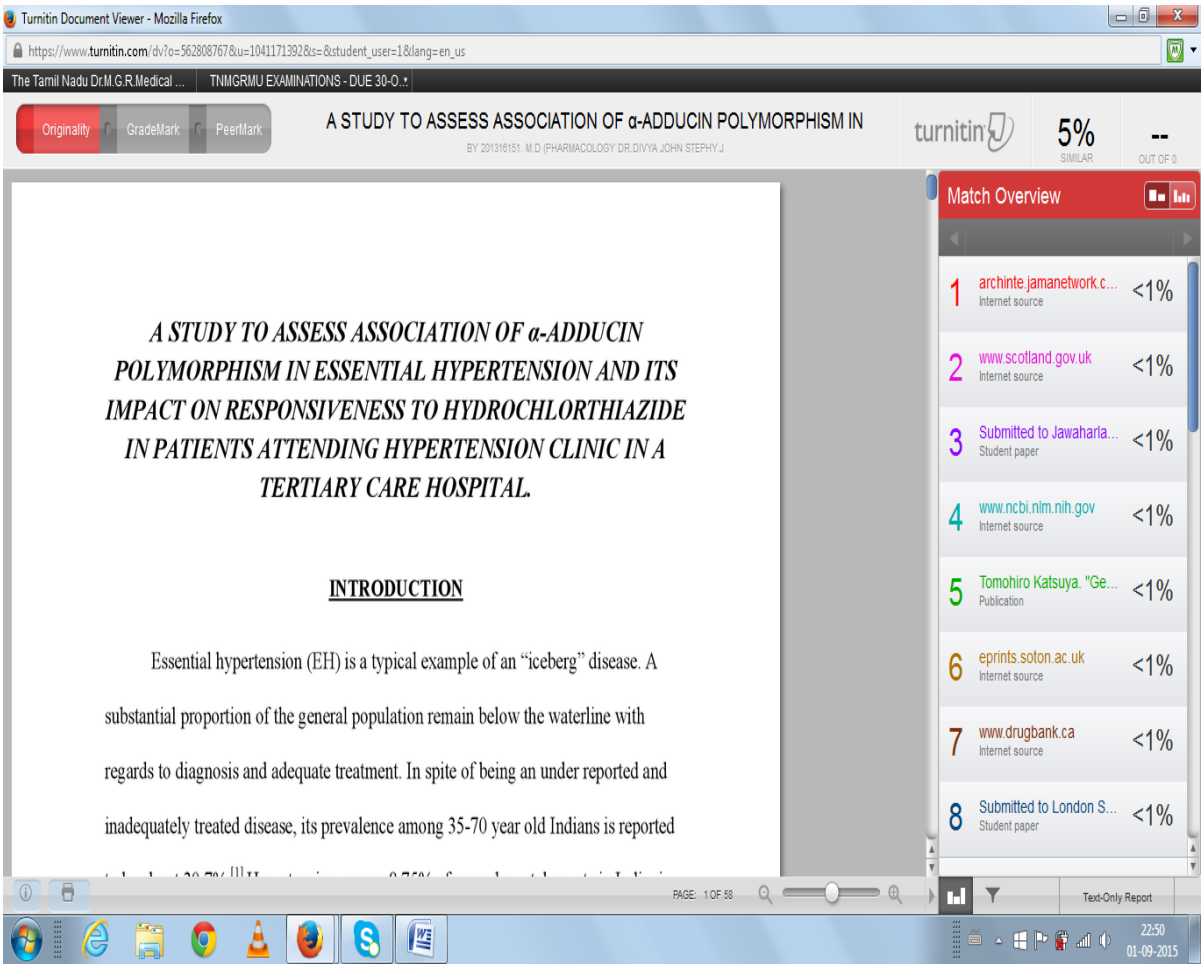
இடம் :
தேதி :

நோயாளியின் கையொப்பம் /
பெருவிரல் கைரேகை :

இடம் :
தேதி :

ANTI-PLAGIARISM REPORT GENERATED VIA TURNITIN





APPROVAL CERTIFICATE FROM INSTITUTIONAL ETHICS COMMITTEE

INSTITUTIONAL ETHICAL COMMITTEE
GOVT.KILPAUK MEDICAL COLLEGE,
CHENNAI-10
Ref.No.3182/ME-1/Ethics/2014 Dt:08.05.2014.
CERTIFICATE OF APPROVAL

The Institutional Ethical Committee of Govt. Kilpauk Medical College, Chennai reviewed and discussed the application for approval "A Study to assess association of α-adducin polymorphism in essential hypertension and its impact on responsiveness to hydrochlorthiazide in patients attending hypertension clinic in a tertiary care hospital" – For Project Work submitted by Dr.Divya John Stephy.J, MD (Pharm), PG Student, KMC, Chennai-10.

The Proposal is APPROVED.

The Institutional Ethical Committee expects to be informed about the progress of the study any Adverse Drug Reaction Occurring in the Course of the study any change in the protocol and patient information /informed consent and asks to be provided a copy of the final report.

GOVT. KILPAUK MEDICAL COLLEGE
30 MAY 2014
Kilpauk, Chennai-10.

30/5/14.
CHAIRMAN,
Ethical Committee
Govt.Kilpauk Medical College,Chennai